ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

ANAEROBIC TREATMENT OF DILUTED WASTE FROM POULTRY INDUSTRY AND QUANTIFICATION OF MICROBIAL COMMUNITIES

M.Sc. THESIS

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Department of Environment Engineering

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

SEYRELTİK KANATLI HAYVAN ENDÜSTRİSİ ATIKLARININ HAVASIZ ARITIMI VE MİKROBİYAL TOPLULUKLARIN KANTİTATİF ANALİZİ

YÜKSEK LİSANS TEZİ

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Aigerim Jaxybayeva, a M.Sc.. student of ITU Institute of Sciency and Technology student ID 501101723, successfully defended the thesis entitled "ANAEROBIC TREATMENT OF DILUTED WASTE FROM POULTRY INDUSTRY AND QUANTIFICATION OF MICROBIAL COMMUNITIES"
which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my future,

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May 2014

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ABBREVIATIONS

ASB : Anaerobic Sludge Bed
BOD : Biochemical Oxygen Demand
DGGE : Denaturing gradient gel electrophoresis
FAN : Free ammonia nitrogen
FISH : Fluorescent in situ hybridization
GDNA : Genomic DNA
GHG : Greenhouse Gas
HRT : Hydraulic retention time
OLR : Organic loading rate
PCR : Polymerase Chain Reaction
PMP : Potential methane production
Q-PCR : Quantitative real-time PCR
Qslurry : Flow-rate of the slurry
SMA : Specific methanogenic activity
Soluble COD : Soluble chemical oxygen demand
TAN : Total ammonia nitrogen
Total COD : Total chemical oxygen demand
TKN : Total Kjeldahl nitrogen
TS : Total solids
TSS : Total suspended solids
VFA : Volatile fatty acids
VS : Volatile solids
VSS : Volatile suspended solids

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ANAEROBIC TREATMENT OF DILUTED WASTE FROM POULTRY INDUSTRY AND QUANTIFICATION OF MICROBIAL COMMUNITIES

SUMMARY

Anaerobic digestion is widely known as a natural process, which converts the biomass (plants, animals or their wastes) to energy and which is now used as one of the most appropriate waste treatment alternatives owing to pollution control and energy recovery. In anaerobic digestion, naturally occurring microorganisms are used to breakdown organic materials and produce biogas, a mixture of mainly methane and carbon dioxide. Many agricultural and industrial wastes can release undesired methane into the atmosphere, but treatment and recovery of this gas by anaerobic treatment processes reduces this source of atmospheric methane. In addition, biogas can be combusted to produce renewable electricity. Hence, anaerobic treatment is a preferred waste treatment process since it produces, sufficiently than consumes, energy and the products of anaerobic digestion have value and can be sold to offset treatment costs.

In this respect, poultry litter, a combination of accumulated chicken manure, feathers, and bedding materials (obtained from broiler houses), are ideal candidates for anaerobic digestion because they contain high levels of easily biodegradable materials. Since animal wastes contain high ammonia concentrations, these wastes have high buffering capacities. Moreover, all animal wastes have lower TS contents (3-5% for the piggery wastes and 6-9% for the cattle wastes) than the organic fraction of municipal solid wastes. Besides, poultry manure contains significant concentrations of organic nitrogen due to the presence of high levels of protein and amino acids. Of the nitrogen in fresh manure, 60-80% is typically in the organic form, such as urea and protein. Depending on the environmental conditions, a large percentage of this organic nitrogen (40-90%) is converted into ammonia within a year. Thus, during anaerobic digestion of poultry manure, the concentration of endogenous ammonia nitrogen might rise considerably.

In this study, anaerobic treatability of chicken (laying hen) manure was evaluated in a laboratory scale Anaerobic Sludge Bed (ASB) reactor inoculated with the granular sludge source already adapted to chicken manure and the reactor was operated at ambient temperature in order to avoid external heating up to mesophilic temperatures. Since heat requirement for raising the temperature of the incoming feed for anaerobic digestion is eliminated, energy recovery from anaerobic treatment of chicken manure could be realized with less operating costs. Reactor was fed daily with the diluted chicken manure (with an influent feed ratio of 1 kg of fresh chicken manure to 6 liter of tap water) at different HRT values (~8.6-26 days). The treatment performance of the ASB reactor was evaluated with the assessment of the biogas production and some conventional parameters like total and soluble chemical oxygen demand (Total COD and Soluble COD), total suspended solids (TSS), nitrogen, pH and alkalinity changes. Additionally, operational temperature and the produced biogas results were also recorded daily. In microbiological studies, quantification analysis of bacteria, archaea, and methanogens have been done using real time PCR method.

Results indicated that average daily biogas productions were 2365 and 2140 mL/day for Slurry-I and Slurry-II, respectively at the same HRT ~ 13 days. Besides, average Total COD removal efficiencies in the ASB reactor were around 89% and 90% for Slurry-I and Slurry-II, respectively. On the other hand, average Soluble COD removal efficiencies were about 63% for Slurry-I and 75% for Slurry-II. Regarding TSS and VSS removals, similar results were observed for both slurries. For Slurry-I, TSS and VSS removal efficiencies were ca. 98 and 97%, respectively, whereas they were both 99% for Slurry-II. Microbiological analysis showed a shift in methanogenic community during biogas recovery and as the number of bacterial community decreased, the amount of archaea increased through the effective digestion volume of the ASB reactor. Moreover, the number of methanogens displayed an uptrend like archaeal community. Methanogenic community showed correlation with acetate concentration in both Slurry-I and Slurry-II.

SEYRELTİK KANATLI HAYVAN ENDÜSTRİSİ ATIKLARININ HAVASIZ ARITIMI VE MİKROBİYAL TOPLULUKLARIN KANTİTATİF ANALİZİ

ÖZET

Günümüzde ekosistemin bozulmasına sebep olan unsurlardan olan hızlı sanayileşme, tüketim miktarlarındaki artış ve yoğun şehirleşme gibi sebeplerden dolayı önemli çevre sorunları da ortaya çıkmaktadır.

Son yıllarda atıksuların arıtımı için uygulananteknolojilerdeki gelişmelere paralel olarak; oksijensiz arıtma teknolojisi, kuvvetli organik madde içeren tarımsal atıksuların ve hayvan çiftliklerinden kaynaklanan atıksuların arıtıldığı tesislerde ortaya çıkan arıtma çamurlarının arıtılmasında yoğun olarak uygulanmaktadır. Hayvan çiftliklerinden kaynaklanan atık suların kirlilik potansiyeli de yüksektir. Bu tür endüstriyel faaliyetlerden oluşan atıksular, arıtılmadan alıcı ortamlara verildiği zaman yüksek miktarda kirlilik oluşturmaktadır. Hayvancılık işletmelerinin ortaya çıkardığı kirlilik kaynakları, endüstriyel ve kentsel kirlilik kaynaklarından da daha geniş alanlara yayılabilmektedir. Bunun nedeni ise, noktasal kirlilikten farklı olarak, su ya da genel olarak biyosferdeki kirliliğin tespit edilmesinin daha zor olmasıdır. Yayılı kirlilik kaynakları (gübreler, hayvansal atıklar vb.) yeraltı sularına veya yüzeysel sulara ulaşarak su kaynaklarının kalitesini bozmakta ve kullanılamaz hale getirmektedir.

Anaerobik arıtma prosesleri, atıklardan enerji geri kazanımını sağlayarak, atıkların nihai olarak uzaklaştırılmaları açısından en çok tercih edilen bir biyolojik arıtma teknolojisidir. Anaerobik şartlarda arıtma ile atıksuların içerisindeki organik maddeler enerji amacı ile kullanılarak biyogaza dönüştürülebilir. Böylece, hem atıksuyun kirlilik yükü azaltılır, hem de yenilenebilir bir enerji kaynağı olan biyogaz üretimi gerçekleştirilebilir.

Son yıllarda hızlı bir ivme ile gelişen tavukçuluk sektörü, bazı çevresel problemleri de beraberinde getirmektedir. Özellikle beyaz et tüketiminin, dünya çapında olduğu gibi, Türkiye'de de son yıllardaki hızlı artışı; hayvancılık sektöründe büyük oranda et

ve yumurta işletmelerinin çoğalmasına neden olmuş ve bu durum Türkiye'de, kümes hayvanlarından kaynaklanan katı ve sıvı atıkların önemli oranda artmasına sebep olmuştur.

Çevre sorunlarına neden olan tavuk çiftliklerinin atıkları, aynı zamanda önemli bir ekonomik potansiyel taşımaktadır. Hayvansal atıkların çoğu gübre ve yem üretimi gibi amaçlarla kullanılmaktadır. Kanatlı hayvanlardan kaynaklanabilecekdışkı miktarına bakıldığında; ortalama olarak bir kümes hayvanından yılda 0,022 ton gübre ortaya çıkmaktadır. Böylece, Türkiye'de yılda üretilen yaklaşık 7 milyon ton civarındaki kanatlı hayvan gübresinin ciddi çevre problemlerine yol açacağı ön görülmektedir.

Tavuk gübresinin karakteristik özelliği katı madde içeriğinin %10-30 arasında, NH_4-N konsantrasyonlarının ise oldukça yüksek (~ 8 g/L) olması sebebiyle - yüksek miktarlarda protein ve amino asit içerikleri - yüksek konsantrasyonlarda organik azot içermektedir. Anaerobik arıtma uygulaması için uygun bir substrat olan tavuk gübresinden yüksek miktarlarda metan gazı elde edilebilmektedir. Havasız arıtma uygulaması, çevre kirliliğinin önlenmesi ve enerji ihtiyacı gibi hususların önemli bir kısmına çözüm sunmaktadır.

Türkiye'de de havasız arıtma konusunda yapılan araştırmalar gün geçtikçe gelişim göstermektedir. Havasız arıtma, hızlı ve fizibıl bir şekilde organik atıkların yönetimine/arıtımına çözüm sunmakta olup; bu sebeple oldukça fazlauygulama potansiyeline sahiptir. Fakat yeni teknolojilerin kullanılmasının oldukça pahalı olduğu Türkiye'de havasız arıtma uygulamalarıhenüz tam olarak yeterli değildir. Bu kapsamda, Havasız Çamur Yataklı Reaktör (HÇYR)'ler gibi yüksek hızlı havasız sistemlerin, özellikle hayvan atıkları gibi yüksek organik madde içeriğine sahip atıkların arıtımında uygulamaları da literatürde henüz çok kısıtlıdır.

Genel olarak havasız çamur yataklı reaktörler; havasız filtrelerdeki sentetik dolgu malzemesinin pahalı olması, tıkanma, kanallanma, büyük debilerdeki aşırı yük ve biyokütle kaybı gibi dezavantajları olmayan, içerisinde yatak malzemesi bulunmayan, ayrı bir mekanik karıştırma ve harici bir çöktürme birimine ihtiyaç duyulmadığı için yüksek hızlı bir sistemlerdir. Arıtma, reaktörün alt kısmında bulunan granüler yapıdaki çamur yatağı ile bunun üst kesimindeki çamur örtüsünde gerçekleştirilmektedir.

Türkiye'de inek, koyun ve kümes hayvanları sayısının yaklaşık olarak sırasıyla 13, 30 ve 265 milyon olduğu göz önüne alınırsa; yıllık atık kapasiteleri sırasıyla 128, 25, 8 milyon ton civarında hesaplanmaktadır. Yıllık toplam katı madde miktarları (TKM) ise 16.2, 6,1 ve 1,9 milyon ton değerlerindedir. Katı maddenin metana dönüşüm oranının 0,150 m^3 CH_4/kg TKM olduğu kabul edilirse; metan üretiminin yılda yaklaşık 1,87 milyar m^3 olduğu hesaplanabilir. Buna göre yıllık enerji geri kazanım potansiyeli (%60 geri kazanım olduğu kabul edilirse) 5,43 milyon MW-saat olmakta ve bu değer 620 MW'lık bir enerji tesisine karşılık gelmektedir.

Tavuk gübresi gibi yüksek kirlilikteki atıkların HÇYR sistemleri ile arıtımları sayesinde yüksek oranda organik madde gideriminin yanında çok yüksek miktarlarda biyogaz geri kazanımı da mümkün olabilmektedir. HÇYR sistemlerinde biyogaz geri kazanımı değerlendirilirken, reaktör içerisindeki biyokütlenin özelliklerinin de mutlaka incelenmesi gerekmektedir. Bu kapsamda, son yıllarda uygulamaları oldukça artmış olan moleküler tekniklerden yararlanılmaktadır. HÇYR sistemleri gibi yüksek hızlı havasız reaktörlerin en önemli özelliklerinden biri, bu reaktörlerde yüksek miktarlarda granül anaerobik çamurun bulunabilmesidir. Diğer havasız sistemlerin yatak malzemesi boşluklarında kısmen gerçekleşen granülasyon; HÇYR sistemlerinde herhangi bir dolgu malzemesi olmadığı halde oluşturulabilmektedir. Çamur hacim indeksi (ÇHİ) < 40 mL/g ve metan verimi yüksek olan bu çamurdaki granüllerin ortalama çapları 1-2 mm olup bazı hallerde 5 mm'ye kadar artış gösterebilmektedir.

Reaktördeki granül çamurun hem farklı tipte anaerobik bakterilerin oluşumuna hem de mikrobiyal yapısı ile bileşimine dikkat edilmesi gerekmektedir. Granüller genellikle yapısı kompleks katmanlıdır. Dış yüzeyde, tam olarak fermantatif bakteriler ve hidrojenotrofik metanojenler bulunur. İç tabakada ise asetik asit kullanan (asetiklastik) metanojenler ve hidrojen üreten bakteriler bulunmaktadır. Bunun yanında, farklı türlerde anaerobik bakterilerin, granüller içerisinde birlikte bulunabilmektedir.

Bu tez çalışmasında, 6,45 L hacime sahip olan laboratuvar ölçekli bir HÇYR kullanılmıştır. Reaktör, 1 m yükseklikte ve 90 mm çapında pleksicam kolonlardan oluşmuştur. Reaktör; konik bir giriş kısım silindirik bir gövde, bir gaz-sıvı-katı ayırıcı bölme ve bir çıkış savağı olmak üzere 4 ana parçadan meydana gelmektedir. Sisteminin farklı noktalarından kolay bir şekilde numune alabilmek ve reaktörün

etkili hacmi boyunca biyokütle değişimi hakkında bilgi sahibi olabilmek amacıyla silindirik gövde üzerine 5 adet numune alma musluğu monte edilmiş. Çalışmada, iki farklı çiftlikten gelen tavuk atığı numunelerinden yararlanılmıştır. Laboratuvara getirilen atık daha sonra +4°C'de muhafaza edilmiştir. Çalışmanın işletmeye alma süresinde, daha önceden tavuk atığına adapte olmuş olan granül aşı çamuru kullanılmıştır. Reaktör, doğal ortam (oda) sıcaklığında işletilerek, herhangi ilave bir ısıtma uygulanmamıştır. Besleme sırasında reaktörün yoğun substrat ile tıkanmaması amacıyla ham tavuk atığı 1+6 oranında seyreltilmiş ve bu karışım 4,00 mm çaplı elekten geçirildikten sonra reaktörün giriş kısmından yukarı akışlı olacak şekilde sisteme beslenmiştir. Reaktörün her gün seyreltilmiş tavuk atığı beslenmesine dikkat edilmiş ve çalışma süresince HÇYR farklı hidrolik bekletme süreleri ile işletilmiştir (~13-26 gün). Reaktörde oluşan biyogaz günlük olarak bir gaz metre yardımıyla kaydedilmiştir. Reaktörde gerçekleşen giderimlerin araştırılmasıiçin pH, alkalinite, toplam ve çözünmüş KOİ, AKM, UAKM gibi konvansiyonel parametreleri düzenli olarak ölçülmüştür. Ayrıca, mikrobiyolojik aktivitelerin çeşitliliği ve miktarı hakkında da çalışmalarda bulunulmuştur.

Tez çalışmasının başında, çalışmanın esası ve önemi hakkında detaylı bilgi verilmiştir. Çalışma kapsamında literatürdeki benzer araştırmalar hakkında bilgiler de verilmiştir. Havasız arıtmaya etki eden faktörler ile havasız arıtmada kullanılan sistemlerden kısaca bahsedilerek çalışmada kullanılan HÇYR sistemleri hakkında genel bilgilere yer verilmiştir. Havasız arıtma sürecinde mikroorganizmaların rolü hakkında literatür bilgilerine de yer verilmiştir. Ayrıca hayvan atıklarının, özellikle tavuk atıklarının karakteristik özellikleri hakkında bilgi sunulmuştur.

Tez çalışmasının 'Materyal ve Yöntem' kısmında, çalışmada kullanılan tavuk atığı ve granül aşı çamurunun özelliklerinden bahsedilmiştir. İki farklı seyreltik tavuk atığının karakterizasyonu, HÇYR'nin işletme koşulları, yapılan deneylerde kullanılan ekipmanlar ve analitik yöntemler hakkında da bilgi verilmiştir.

Çalışmanın 'Sonuçlar ve Tartışma' kısmında, günlük olarak ölçülen işletme sıcaklık değerleri ile biyogaz üretimleri gibi veriler de verilmiştir. Granül aşı içeren HÇYR'den elde edilen arıtma verimi sonuçları konvansiyonel parametrelerde izlenen değişimleri içerecek şekilde çizelgeler ve grafikler halinde sunulmuştur. Ayrıca, reaktördeki çamur yatağına ait mikrobiyolojik çalışmalar da gerçekleştirilerek; toplulukların miktarları tespit ve tayin edilmiştir. Anaerobik arıtma proseslerinde

kompleks organik bileşiklerin metan gazına dönüştürülmesinde, çeşitli mikroorganizma grupları yer almaktadır.

Çalışmanın sonuç kısımında, doğal ortam sıcaklığında (herhangi bir ilave ısıtma uygulanmaksızın) iki farklı seyreltik tavuk atığı ile beslenen ve farklı hidrolik bekleme süreslerinde işletilen HÇYR'de gerçekleşen biyogaz üretimleri giderilen TKOİ, parametresi açısından analiz edilmiştir. Buna göre, bekletme süresinin yaklaşık 13 gün olduğu deney düzeneğinde günlük ortalama biyogaz üretim miktarları Atık I için 2365 mL/gün ve Atık II için 2140 mL/gün'dür. Bunun yanında, havasız çamur reaktörde toplam KOİ giderim verimleri Atık I için %89, Atık II için ise %90 bulunmuştur. Diğer yandan çözünmüş KOİ giderim verimleri Atık I'de %63, Atık II'de %75'tir. Her iki çamur için de AKM ve UAKM giderim verimleri benzer bulunmuştur. Atık I'de AKM giderimi %98, UAKM %97 olarak ölçülmüş, bu değer Atık II 'de %99 olarak bulunmuştur.

1. INTRODUCTION

Many authorities have been investigating alternative energy sources because of the high cost of energy obtained from the fossil fuels. In recent years, producing energy through biomass, is among the most promising renewable energy options, and is vigorously pursued because of its positive environmental implications. In this respect, applications of anaerobic digestion for animal manure (e.g. the manure from poultry industries) treatment have improved significantly in all around the world.

1.1 Signifacancy of this Study

In recent years, backyard poultry farms gave way to the modern world, with two main branches of products: meat and egg producing. Nowadays particularly in the developing countries, poultry products have taken significant place in food markets. However, with a high production capacity and with the development of the poultry sector, the wastes arising from this sector have started to pose significant environmental problems. Before the development of the poultry sector, chicken manure has been used as a fertilizer for agricultural purposes. The increasing amounts of the chicken wastes and the potential pollution threat to the environment (i.e. surface and subsurface water, soil and air) need appropriate management and disposal methods to be applied.

1.2 Aim and Scope of the Study

The major objective of this study was to investigate the removal efficiency and biomethane recovery potential of the diluted manure from the poultry industry (laying hen manure) during anaerobic treatment by a laboratory-scale ASB reactor under ambient operating temperatures. In this scope, the ASB reactor has been operated at room temperature without any additional heating with a hydraulic retention time of ca. 13 and 24 days. The reactor has been fed with the diluted chicken manure (with the ratio of 1 kg of raw chicken manure to 6 L of tap water) on

daily basis. The treatment performance of the ASB reactor was assessed by the biogas produced and the changes in conventional pollution parameters. Moreover, identification/quantification of the microbial communities present in the granular inoculum was the other objective of this study. In this scope, number of bacteria, archaea and methanogens involving in the digestion process was also quantified.

In the scope of this study the following studies were performed;

- Investigation of anaerobic treatability of the diluted chicken manure in a lab-scale ASB reactor at ambient operating temperature,
- Evaluation of the biogas production rate regarding treatment performance of the ASB reactor,
- Quantification of the microbial communities present in the granular sludge along the ASB reactor.

2. LITERATURE REVIEW

2.1 Anaerobic treatment

Anaerobic digestion is the oldest biological wastewater treatment process that has been used for more than one hundred years. It is now used around the world as a type of treatment for municipal, agricultural and industrial wastes.

By definition, anaerobic means "without air" and anaerobic digestion means the breakdown of organic material by anaerobic microorganisms in the absence of oxygen (Beatriz , 2010). Anaerobic digestion of solid structures results in significant decreases in the levels of solids in the digester sludge and leads to the formation of natural gas, or biogas as set out in Figure 2.1 below.

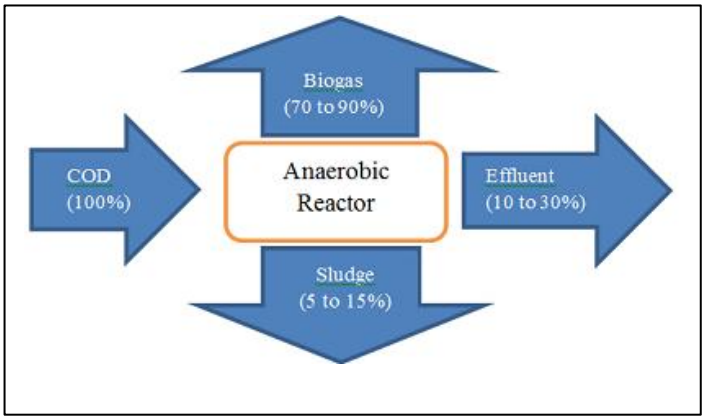

Figure 2.1: Biological conversion in anaerobic system (Augusto, 2007).

Anaerobic biodegradation involves complex metabolic interactions between various groups of microorganisms. In many sources of literature anaerobic digestion is usually described as a four-stage process: hydrolysis, abiogenesis, acetogenesis and methanogenesis or alternatively, as a three-stage process that includes hydrolysis or liquefaction, acidogenesis and methanogenesis. This multistep nature of anaerobic biodegradation is shown in Table 2.1. Three basic bacteria groups (acidogens, acetogens, and methanogens) are recognized in this process. To ensure process continuity and stability, products formed from the activity of a particular bacteria group serve as substrate for another bacteria group.

Table 2.1 : The three stages of anaerobic digestion of solids (Gerardi, 2003).

Stage	Activity
First	Hydrolysis: Solubilization of particulate and colloidal wastes
Second	Acid forming: Conversion of soluble organic acids and alcohols to acetate, carbon dioxide, and hydrogen
Third	Methanogenesis: Production of methane and carbon dioxide

Methane-forming bacteria are strict anaerobes and are extremely sensitive to changes in alkalinity, pH and temperature. Therefore, operational conditions in the digester must be periodically monitored and maintained within optimum ranges. These conditions include gas composition, hydraulic retention time (HRT), oxidation-reduction potential (ORP) and volatile acid concentration as in Table 2.2 below.

Table 2.2 : The three stages of anaerobic digestion of solids (Gerardi, 2003).

Condition	Optimum	Marginal
Alkalinity, mg/L as $CaCO_3$	1500-3000	1000-1500 3000-5000
Gas composition		
Methane, % volume	65-70	60-65 & 70-75
Carbon dioxide, % volume	30-35	25-30 & 35-40
Hydrolic retention time, days	10-15	7-10 & 15-30
pH	6.8-7.2	6.6-6.8 & 7.2-7.6
Temperature, mesophilic	30-35°C	20-30° & 35-40°C
Temperature, thermophilic	50-56°C	45-50° & 57-60°C
Volatile acids, mg/L as acetic acid	50-500	500-2000

Anaerobic processes involve low-cost technologies with advantages and disadvantages regarding operation and maintenance as illustrated in Table 2.3.

Table 2.3 : Advantages and disadvantages of the anaerobic processes (Augusto, 2007).

Advantages	Disadvantages
• Low production of solids (3 to 5 times lower than in aerobic Processes) • Lower energy consumption and very low operational costs • Low land requirements • Low construction costs • Production of methane, a highly calorific fuel gas • Possibility of preservation of the biomass, with no reactor feeding, for several months • Tolerance to high organic loads • Application in small and large scale • Low nutrient consumption	• Inhibition by large number of compounds • Slow process start-up in the absence of adapted seed sludge • Need for some form of post-treatment • Complex biochemistry and microbiology of the process that requires further studies • Possible generation of bad odours, although they are controllable • Possible generation of effluents with unpleasant aspect • Unsatisfactory removal of nitrogen, phosphorous and pathogens

2.2 Factors affecting anaerobic treatment

Multiple factors affect the design and performance of anaerobic digestion processes. Within the framework of anaerobic environment, different important parameters affect the rates of the three stages of the anaerobic digestion processes. These include pH level and alkalinity, oxidizing agents, nutrients and essential fatty acids, temperature, inhibition and toxic agents. For efficient anaerobic treatment process to take place these parameters must be supported at optimal levels and the production of toxic and inhibitory substances must be depressed. These parameters and their optimal levels are described in the following sections.

2.2.1 pH and alkalinity

Anaerobic bacteria, mainly methanogens, are very sensitive to the acid concentration within the digester and their growth can be inhibited by acidic conditions. Alkalinity is crucial in pH control and in enhancing digester stability. Alkalinity is mainly present in the form of bicarbonates in equilibrium with carbon dioxide gas at a given pH (Gerardi, 2003). Therefore, pH depends on the partial pressure of CO_2 and balance between acid and alkaline components in the liquid phase and moreover can be used as indicator of methanogenic consortium performance (Cheng et al., 2008; Gerardi, 2003; Poliafico, 2007). After gas production, pH is the one of the best indicator of future digester balance (Poliafico, 2007). Initially, methanogenic activity

occurs at pH between 6.2 and 8 with an optimum range between 7.0 and 7.2 (Gerardi, 2003; Poliafico, 2007). It has been determined that an optimum pH value for anaerobic treatment should range between 5.5 and 8.5 (RISE-AT, 1998). As the methane stage is the rate-limiting stage, pH should be kept near 7. The optimal pH for bacterial growth of anaerobic organisms is in the range of 6.5 to 7.5 (Sakar, et al., 2009).

2.2.2 Oxidizing agents

In order to provide a stable anaerobic treatment process, the operating environment must be absolutely free of oxygen. This is because chemically bonded oxygen can negatively affect the treatment process. Some substances such as NO_3^-, H_2O_2, SO_4^{-2} and HS^- can also adversely affect the efficiency of treatment systems.

2.2.3 Nutrients and essenitial fatty acids

All microorganisms need macro and micro-nutrient for basal metabolism and grow under both aerobic and anaerobic conditions. Carbon, oxygen, nitrogen, hydrogen and phosphorus are the main components of organic wastes and microbial cell material is approximately 50, 20, 12, 8 and 2 percent of those elements, respectively (Gerardi, 2003). All these elements with iron, magnesnesium, calcium, nickel, sodium, barium, tungstate, molybdate, cobalt and selenium are very necessary for the formation of methanogens (Henze et al., 1983). Some of these elements such as selenium, nickel and tungsten are vital in the enzyme systems of acetogenic and methanogenic microorganisms (Stronach et al., 1986). Moreover, sulphur is required to synthesize vital proteins in metabolic and anabolic pathways (Madigan et al., 2008). These are commonly considered as macro nutrients and must be present in digester feedstock at 10^{-4} M.

Due to the many complex interactions between various constituent populations of the microbial consortium, a number of factors can disturb anaerobic digestion. Excessive volatile fatty acids (VFA) accumulation can inhibit methanogenesis, though high hydrogen levels can inhibit propionate- butyrate-degrading and acetogens (Magbanua , et al., 2001).

In anaerobic treatment is often affected by volatility fatty acids (VFA). Usually, change in VFA presentation is the most sensitive parameter because the primary

cause of anaerobic digester failure roots from the imbalance between acidogenic, acetogenic and methanogenic organisms (Lahav and Loewenthal , 2000). The fraction of undissociated VFA can increase when the pH decreases due to VFA production by acetogens. When the amount of undissociated VFA residues high for continued periods, methanogens are slowly wiped out and acetogens predominate in the bioreactors. If sufficient buffering capacity is existing, the eventual production of VFA during occasional overloading will not decrease the pH and the undissociated VFA fraction will be too small to significantly disturb the methanogens. With the production of VFA, little COD removal is achieved. In order to obtain an effective COD removal and methane production, it is necessary to control VFA accumulation during all period of treatment (Sakar, et al., 2009).

2.2.4 Inhibition and toxic agents

Many literature sources on anaerobic digestion shows considerable variation in the inhibition or toxicity levels reported for most substances. The major reason for these variations is the complexity associated with anaerobic digestion process where mechanisms such as antagonism, synergism, acclimation and complexing can all significantly affect inhibition (Cheng, et al.,2008).

Toxic materials such as fungicides and antibacterial agents can have an adverse effect on anaerobic digestion. While the anaerobic process can handle small quantities of toxic materials without difficulty, it is important to place the storage containers for fungicides and antibacterial agents at locations that will not discharge to the anaerobic digester (Burke, et al., 2001).

Several materials can cause an inhibitory reaction. The materials of greatest concern are light metal cations, ammonia, sulfide and heavy metals. Sulfate, for example, interferes with methane production by providing an alternate electron acceptor while sulfide exerts an oxygen demand that reduces the amount of COD stabilized. Ammonia toxicity is another example of major concern for anaerobic treatment of wastewaters containing high concentrations of total ammonia nitrogen. The free ammonia (or unionized ammonia, NH_3) is also considered to be toxic for methanogenic bacteria.

Many organic compounds are also inhibitory to methanogens (Grady et al., 1999). Methanogenesis is generally the most sensitive step to inhibitory or toxic material

although all groups involved in process can be affected (Speece, 1996). Inhibition of methanogenesis is generally indicated by reduction of methane production and increased concentration of volatile acids.

2.2.5 Temperature

The operating temperature of an anaerobic digestion system significantly affects the process performance. This is because during anaerobic digestion, gas production rate is affected by temperature. Anaerobic digestion process therefore needs to be carried out in the presence of a delicately balanced population of various bacteria that can be very sensitive to changes in temperature. Figure 2.2 shows the relationship between the growth rate of various microorganisms and temperature.

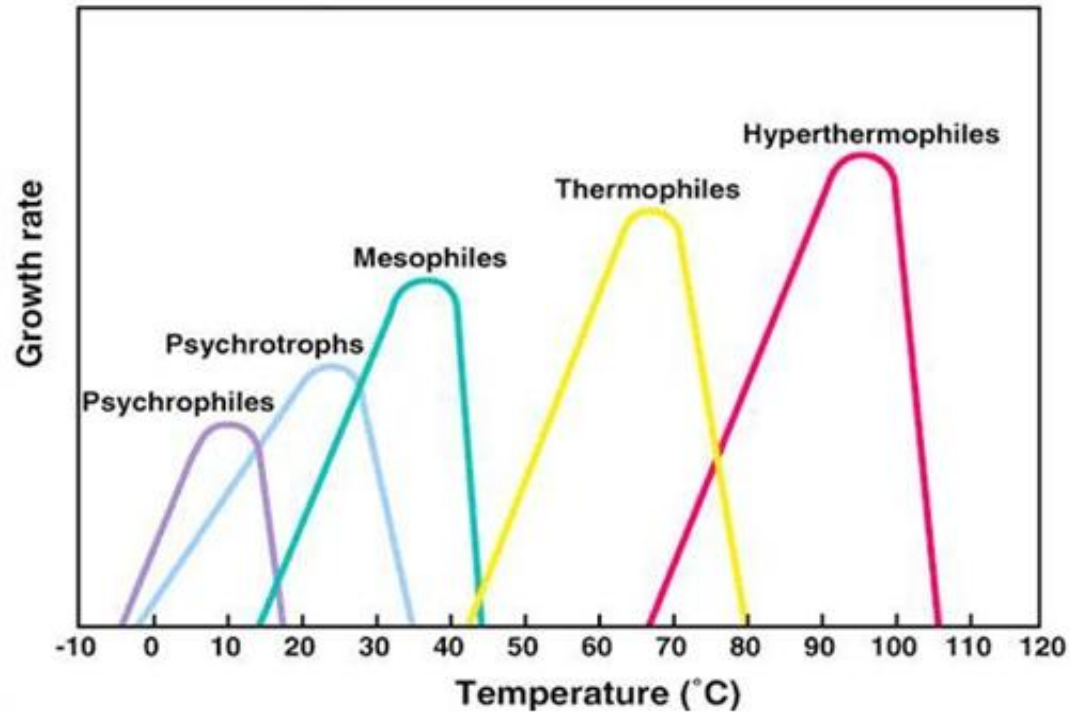

Figure 2.2 : Effect of temperature on microbial growth rate (Rittman and Mccarty, 2001).

Traditionally, anaerobic processes have been carried out under mesophilic conditions although anaerobic digestion can occur even at room temperature. It is important to note that maintaining a constant digester temperature at around 35°C will improve the digester performance.

Three main different temperature ranges are distinguished in technical applications as it is shown in Figure 2.2:

(1) psychrophilic (or cryophilic) temperatures from 10 to 20° C;

(2) mesophilic temperatures from 20 to 40°C; and

(3) thermophilic temperatures from 40 to 60°C.

An extensive scientific research has been carried out, examining the relationship between digester temperature and growth of micro-organisms. Sakar, et al. (2009) showed that temperature affects activity and growth of micro-organisms with methanogenic bacteria being more sensitive to changes in temperature than other micro-organisms in anaerobic digesters. At temperatures between 40 and 50°C, methanogens are inhibited. Optimum biogas production occurs at 35 and 55°C for mesophilic and thermophilic organisms respectively (Angelidaki et al., 2007), which has been confirmed by Verma (2002) who concluded that mesophilic and thermophilic ranges mainly provide optimum treatment conditions for an effective COD removal and methane production in anaerobic treatment.

Mesophilic and thermophilic conditions present different reactor design and operational advantages and drawbacks. During thermophilic digestion, both greater destruction of pathogens and higher substrate degradation (and biogas production) can be achieved (Chen et al., 2008; Gerardi, 2003; Poliafico, 2007).

High temperature allows higher rates of microbial metabolic activity, leading to a shorter retention time required to achieve a given level of solids destruction and a good degree of inactivation of pathogenic organisms (Bernard , et al., 2000). But thermophilic conditions require a large amount of heat energy that reduces the net energy production (El-Mashad et al., 2004).

Many researches have suggested that the gas production during anaerobic digestion is correlated with temperatures. However, different results show that temperature had no effect on the methane yield of beef cattle manure between 30 and 60 ºC. Other researchers suggest that an increase of the temperature results in the reduction of the biogas yield due to the increased inhibition of free ammonia (NH_3) which increases at elevated temperatures (Navickas, et al., 2013). Most of the experiments carried out so far were conducted at 30° C, but it is well known that the optimal temperature for mesophilic growth is situated near 40° C. On the other hand, there is a less difference between mesophilic and thermophilic digestion (Sakar, et al., 2009).

Temperature plays a primary role in the selection of both the identity of individual species and the overall bacterial diversity supported by a treatment reactor (Lapara, et al., 2001). As the temperature falls, bacterial activity decreases and biogas production decreases. As the temperature increases some bacteria begin to die, once again biogas production decreases.

2.3 Anaerobic treatment systems

In the beginning the advent of refined anaerobic treatment technologies or the high rate anaerobic digesters, anaerobic treatment referred to "anaerobic digestion" of solids generated in aerobic biological wastewater treatment operations. In other words, anaerobic treatment was primarily used for the stabilization or the liquidification of solid components of sewage with the intention of reducing the amount of solids.

One of the most critical factor in design of the anaerobic treatment systems is selection of the suitable reactor type and structure to maximize metabolic, nonoxidative bioenergy invention. Anaerobic reactors usually classified as low rate and high rate as shown in Figure 2.3.

Low-rate anaerobic bioreactors are unmixed reactors where temperature, SRT, and other environmental parameters are not ordered. The organic loading rate is low, ranges varied between 1 and 2 kg COD/m^3 day. Mostly these reactor configurations are not appropriate for methane production.

High-rate anaerobic systems can treat a very high biomass level. Environmental conditions are well ordered to optimize performance of the reactor. The organic loading rates can change from 5 to 30 kg COD/m^3 day or even higher. High rate anaerobic reactors are more suitable for methane production.

Anaerobic reactors have been in use since the 19th century when Mouras and Cameron developed the automatic scavenger and the septic tank to reduce the amounts of sewerage system (Henze et al., 2008). The first anaerobic reactor Imhoff tank was designed and developed in Germany in 1905 by Karl Imhoff, in this type of tank solids sediments are stabilized in a single tank. In the same period, Buswell started to adopt the same technology for treating liquid wastes and industrial wastewater (Henze et al., 2008).

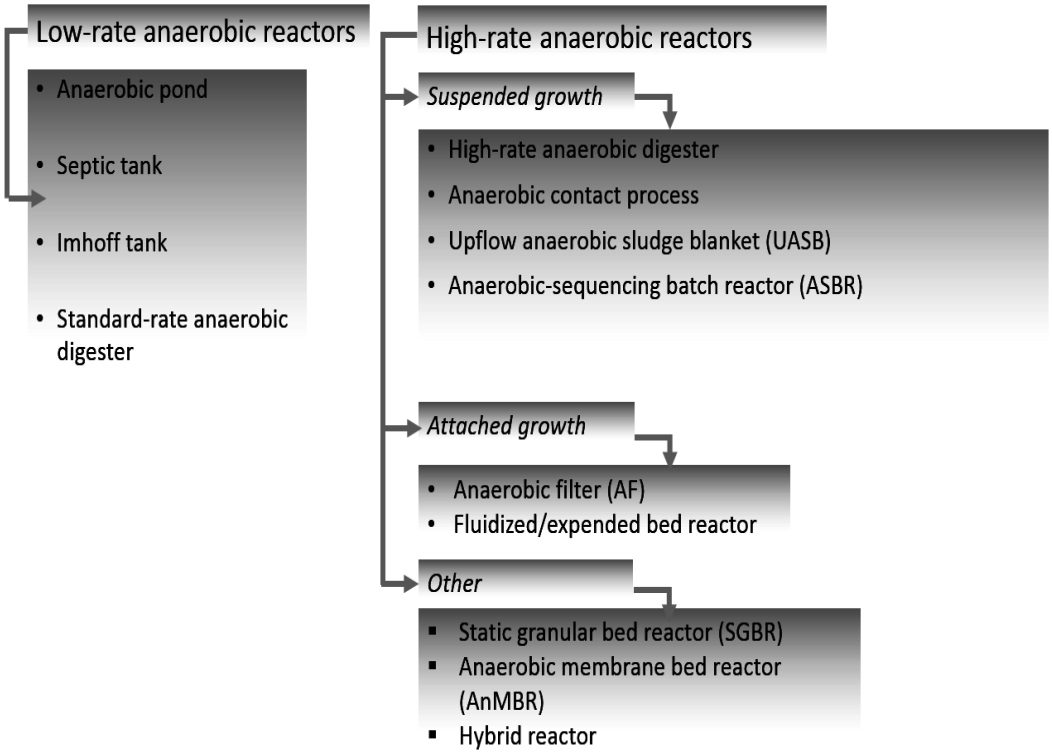

Figure 2.3 : Classification of anaerobic reactors (Khanal, 2008).

It was not until 1955 that anaerobic contact process was developed to treat soluble or dilute organic wastewaters (Calli, 2010). A schematic diagram of Imhoff tank shown in Figure 2.4.

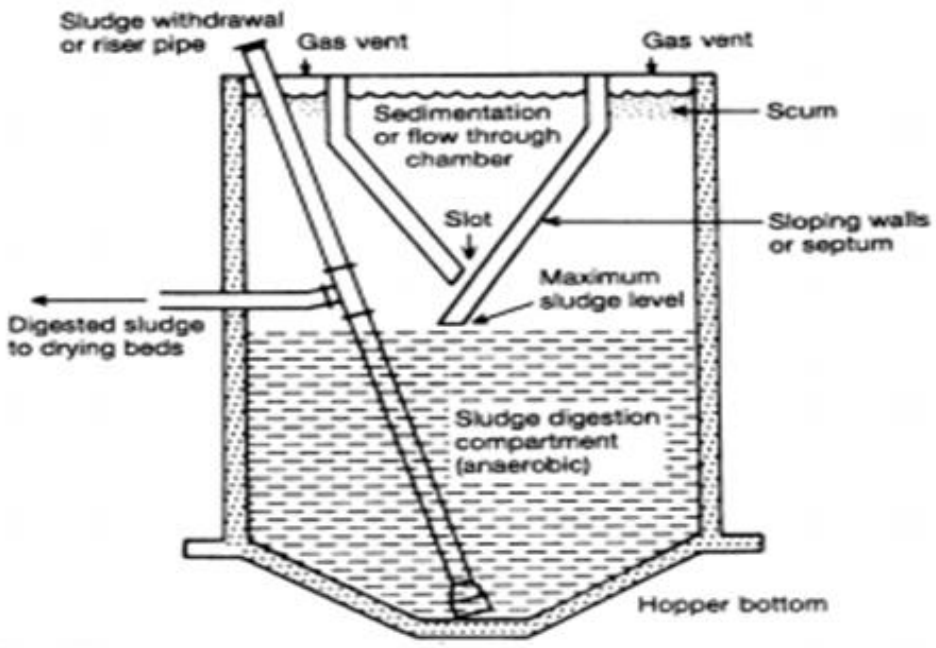

Figure 2.4 : Imhoff tank (Calli, 2010).

2.4 Upflow anaerobic sludge blanket (UASB)

The problem which was associated with anaerobic filters and fluidized bed reactors led to development of unpacked reactors that still incorporate an immobilized form of particulate biomass. The Upflow Anaerobic Sludge Blanket reactor was developed in the seventies by Professor Lettinga and his group at the University of Wageningen in the Netherlands in 1970 (Wang, 1994). The realization of the UASB reactor has been very successful and it has been applied to a wide range of many kinds wastewaters (Schmidt, et al., 1996). Many full-scale UASB treatment plants operated under tropical or subtropical construction and also frequently applied to cold climates as the produced biogas can be used to heat the reactor.

The UASB reactor is usually divided into four compartments: (1) the granular sludge bed, (2) the fluidized zone, (3) the gas-solids separator, and (4) the settling compartment (Schmidt, et al., 1996).Wastewater is introduced from the bottom of the reactor, and it then flows upward through a blanket of active anaerobic sludge, as shown in Figure 2.5. The sludge bed is composed of microorganisms that naturally form granules of 0.5 to 2 mm in diameter that have a high sedimentation velocity and thus resist wash-out from the system even at high hydraulic loads. Treatment occurs as a result of a proper contact of the active sludge with wastewater (Wang, 1994).

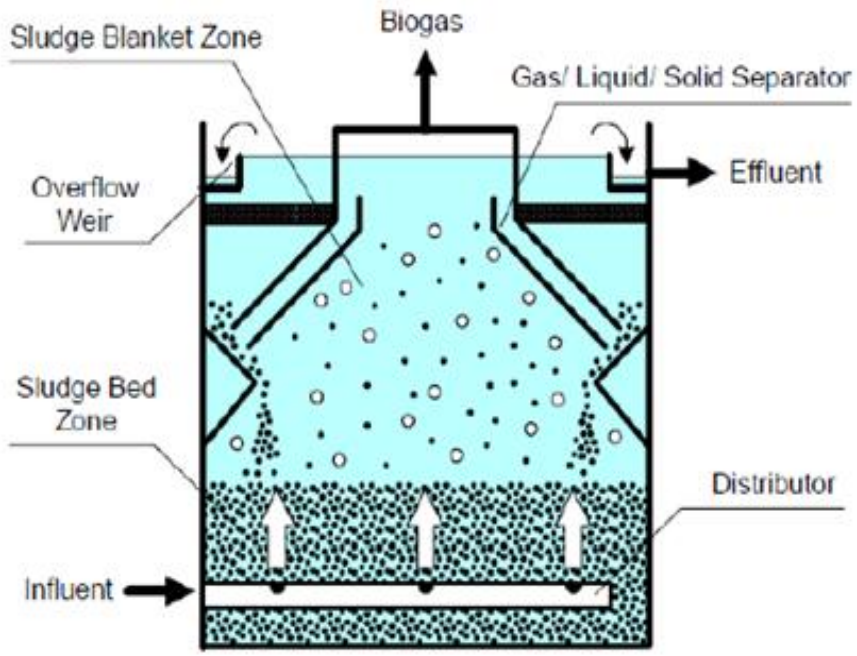

Figure 2.5 : Modified upflow anaerobic sludge blanket (UASB) reactor (Agbalakwe, 2011).

The upward motion of released gas bubbles causes hydraulic turbulence that make available reactor mixing without any mechanical agitation, because the gases produced in the sludge pall become partly entrapped into the sludge. The particles with the attached gas and free gas bubbles become to rise to the top of the treatment reactor. The gas released from the sludge is taken in the gas collection dome located at top of the reactor. Gas is collected in the hoods and removed from the reactor. (Wang, 1994).

UASB reactor can operate at short hydraulic retention times since the sludge retention time is almost independent of the hydraulic retention time. Successful operation under these conditions requires a highly active biomass with good settling abilities (Schmidt, et al., 1996)

The upflow anaerobic sludge blanket reactor is characterized by a reactor containing no packing or any other type of biomass support material. An important feature of this design is the gas-solids separator which at the top of reactor provides an immovable zone, where all suspended solids will settle (Lettinga , et al., 1984). Compared to the other kinds of anaerobic reactors, UASB reactor has exhibited good performance when they are submitted to high values of volumetric organic loading rates. This is particularly useful for reactors in which granular sludge is formed and maintained, even under psychrophilic conditions (Garcia , et al., 1996) .

2.5 Microbiology of anaerobic processes

Bacteria populations arise from individual cells and metabolically comparable populations such as sulfate-and sulfur reducing bacteria involve groups mentioned as guilds. These sets of guilds conducting interdependent physiological processes form microbial communities. Microorganisms also form natural assemblages at air-water interfaces and in suspensions, such as anaerobic digester systems. They better aggregate to form granules or flocks (Davey and O'Toole, 2000).

The degradation of complex organic matter into carbon dioxide and methane during anaerobic treatment needs the collaboration of at least three guilds. The catabolism is initiated by fermentative bacteria producing acids and alcohols that are then readily utilized by acetogenic bacteria. The methanogens bacteria obtain energy from converting acetate, carbon dioxide, and hydrogen to methane on the final stage.

Thus, very efficient cooperation and mutual dependence can occur within a biofilm providing an ideal environment for the creation of syntrophic relations (Schink, 1997).

In anaerobic treatment four different groups of microorganisms cause the degradation of organic matter to carbon dioxide and methane in separate steps. Among these, methanogens produce methane as an inner part of their energy metabolism. Methanogens are belong to *Archaea* and found at large numbers in this group. Methanogens have the ability to use H_2 that plays an important role as regulatory and controls the types of products made by fermentative bacteria. The most significant substrate for methane creation is acetate which is a major product of fermentative metabolism. Acetate using methanogens consist of members of the genera *Methanosarcina* and *Methanosaeta*.

Methanosaeta and *Methanosarcina* are acknowledged to grow by an acetoclastic reaction, producing methane from acetate among the many methanogenic genera. *Methanosaeta concilii* is solely an acetoclastic bacterium and is the only mesophilic species of its genus, other classes being thermophiles. *Methanosarcina barkeri* is metabolically the most versatile of all the mesophilic methanogenic bacteria isolated in pure culture, because it can form methane from H_2 and CO_2 (hydrogenotroph), from methanol and methylamines (methylotroph), and from acetate (acetoclast) **(**Rocheleau et al., 1999). Cells belong to the genus of *Methanosarcina*, known to utilize acetate for methane creation, have been shown to be common in anaerobic reactors (Sorensen et al., 1997). In general, the granules are composed of *Methanosaeta* spp. rather than *Methanosarcina* spp. in high rate anaerobic reactors**.**

2.6 Microbiology of anaerobic granules

The diameter of sludge granules varies from 0.14 to 5 mm and usually have a spherical form. Their cultivated on acidified substrates, such as acetate, are generally smaller than granules grown on acidogenic substrates, glucose (Schmidt, et al., 1996).

In various studies cultures were identified: typical methanogens are *Methanobrevibacter* spp., *methanospirillum* spp., *Methanosaeta* spp. (former

Methanothrix spp.), and *Methanosarcina* spp. syntrophic bacteria are *Syntrophobacter* spp., *Syntrophomonas* spp., and *Pelobacter* spp. Sulfate reducing bacteria are also present (Schmidt, et al., 1996).

Methanosaeta spp. are known to grow on acetate and are filamentous organisms. *Methanosarcina* spp. are also able to grow on substrates such as methanol, methylamines, and sometimes hydrogen and carbon dioxide. *Methanosaeta* spp. have a specifically lower growth rate at high acetate concentrations than *Methanosarcina* spp., and their affinity for acetate is 5 to 10 times higher. These data show that a low acetate concentration in the effluent from a UASB reactor results in a selection for granules dominated by *Methanosaeta* spp. Though, since *Methanosarcina* spp., unlike *Methanosaeta* spp., can grow on numerous substrates, their role in UASB reactors can't be based only on their aptitude to use acetate. It is found that there was no correlation between the effluent concentrations of acetate from UASB reactors and the ratio of *Methanosaeta* to *Methanosarcina*. It was also reported that probably other factors were involved in selection, such as macro and micro nutrients and the hydraulic loading of the reactor (Schmidt and Ahring, 1999).

Commonly, the granules have a multifaceted layered structure. On the surface layer, mostly fermentative bacteria and hydrogenotrophic methanogens exist and the internal layer is engaged by aceticlastic methanogens and H_2-producing bacteria. Besides, the juxtaposition of different types of anaerobic bacteria is detected in granules. (Jianrong et al., 1997).

The adhesion of cells is mainly dependent on the contacts of cell wall, e.g., surface charges. Because of the fact that the arrangement of cells were observed very close (less than 60 nm in distance) *Methanosaeta* usually play an important role in the formation of network of granular sludge (Jianrong et al., 1997).

In the microstructure of the granules the nature of the substrate plays a very important role too. It was stated that granules degrading soluble carbohydrates exhibited a layered structure, while those degrading glutamate exhibited a rather uniform structure (Fang et al., 1994). They also described that *Methanosaeta* was the main basic element in all anaerobic granules which suggests that these filaments likely play an important role in sludge granulation. Numerous different species of

methanogens and acidifiers, acetogens can be represented by filamentous microorganisms in anaerobic digesters. However, one of the most recognized filamentous methanogenic bacteria is the acetoclastic *Methanosaeta.* From filaments longer that 1000 units to short filaments of 5-10 units different morphologies could be observed for this bacterium which is the dominant acetoclastic species below low substrate concentrations (Alves et al., 2000).

2.7 Identificaion of microbial communites in anaerobic processes

Nowadays only a little part of the microbial ecology within these anaerobic treatment systems has been revealed, yet. Molecular tools for study microbiological medium have been used in last decades and are being developed day to day (Amann et al., 1995). Few percent of Bacteria and Archaea have been isolated, but their dynamic in the system and relations between each other are still unknown. Using the molecular tools in the anaerobic treatment systems give the possibilities to find out which microorganisms exist, learn their activities and also to define their numbers. Microbial ecology studies need identification of species based on a comprehensive classification system that perfectly reflect the evolutionary relations between the microorganisms (Pace, 1996).

Microbial communities of anaerobic treatment processes have been examined by classical parameters (such as VSS) or used microscopic or culture-based counts which are informative but may not be adequate (Akarsubasi et al., 2005). There are two identification techniques such as classical identification techniques and molecular identification techniques. Among classical identification techniques, cultivation dependent methods and microscopic analyses can be listed. In molecular identification techniques, immuno detection, membrane lipid fatty acid analysis, ribosomal RNA/DNA based methods and Fluorescent *In-Situ* Hybridization (FISH) methods are gaining importance .

The spatial distribution of methanogens in methanogenic granular sludge has been studied with various immunological techniques, but the distribution of acetogenic bacteria, especially in this sludge, could not be investigated due to the absence of identification methods allowing a differentiation between the groups of bacteria (Harmsen et al., 1996).Since an unexpectedly large number of organisms with unique phenotypes belong almost exclusively to the members of the

domain *Archaea*, a systematic study on whole-cell hybridization of *Archaea* should be performed. It can not be granted that methods optimized for the members of Bacteria will also be appropriate for *Archaea* (Burggraf et al., 1994).

Zuckerkandl and Pauling (1965) indicated that nucleic acids could document evolutionary history. Due to the pioneering studies, nucleic acids, especially 16S rRNA, are the ultimate biomarkers and hereditary molecules probably because of their essential role in protein synthesis, making them one of the earliest evolutionary functions in all cellular life-forms **(**Pace et al., 1986).

2.7.1 Nucleic acid isolation

DNA and RNA isolation are usually the first step of PCR-based methods. The key point in this step is to obtain representative and bias-free samples. Besides, adequate nucleic acid should be retrieved. After the sampling, DNA samples can be kept at -20 ºC until extraction. But in case with RNA, it should be isolated quickly under appropriate conditions. Few methods have been used for the isolation from different kind of samples such as sludge, water, manure, soil, and sediment. Without bias obtaining RNA or DNA quantitatively from all cells in a complex community can be difficult. Generally, mechanical lysis methods have shown less bias than enzymatic lysis methods, leading to the recovery of intact high molecular weight nucleic acids (Talbot et al., 2008).

2.7.2 Polymerase chain reaction

The polymerase chain reaction (PCR) was invented in 1985 and used to amplify defined target DNA, small part of DNA, which is extracted from environmental samples. Because of the specificity, sensitivity, simplicity and speed of the reaction, PCR has been used in a variety of applications in the last decade; such applications include characterizing the structure and expression of genes; identifying of disease-causing genes and pathogens; diagnosing inherited disease prenatally; and DNA fingerprinting in forensics, agriculture, and archaeology. The products of the PCR are analyzed by further techniques such as cloning-sequencing, DGGE or TGGE which have the potential to separate the PCR products originating from different DNA sequences representing populations in the original samples (Strachan and Read, 1999).

The PCR is a chain reaction because newly synthesized DNA strands will act as templates for further DNA synthesis in subsequent cycles. After about 25 cycles of DNA synthesis, the products of the PCR will include, in addition to the starting DNA, about 10^5 copies of the specific target sequence, an amount which is easily visualized as a discrete band of a specific size when submitted to agarose gel electrophoresis.

2.7.3 Quantitative real-time PCR(Q-PCR)

Real-time quantitative PCR, which known as qPCR, combines PCR amplification and detection into a single step. PCR, Q-PCR is applied for exact quantification of the microbial groups as genus, kingdom or family (Strachan and Read, 1999). This method based on the continuous monitoring of some changes of fluorescence in the PCR tube during amplification. Against to traditional PCR, quantification is done based on the exponential phase of amplification in Q-PCR (Malinen et al, 2003). This eliminates need to detect products using gel electrophoresis, and more significantly it enables the method to be correctly quantitative. With qPCR, fluorescent dyes are used to label PCR products during thermal cycling.

Q-PCR reaction products are fluorescently labeled using two strategies:

- TaqMan fluorogenic probes—target-specific oligonucleotides that produce a fluorescent signal only when the target DNA is amplified during Q-PCR.

- SYBR Green I dye—binds to double-stranded DNA and emits fluorescence only when bound.

2.8 Anaerobic treatment of animal manure

Incorrectly managed animal waste can have severe consequences for the environment such as odour problems, attraction of rodents, insects and other pests, release of animal pathogens, groundwater contamination, surface water runoff, deterioration of biological structure of the earth and catastrophic spills (Sakar, et al., 2009).

Manure can be characterized in some ways. Important properties for manure collection, storage, handling and utilization include the solid content and the size of

manure solids (fixed and VS, suspended solids, and dissolved solids). Nitrogen content in manure varies with the type of animal and feed ration, amount of litter, bedding or soil included, and amount of urine concentrated in the manure. Moisture content is also a major consideration. Normally moisture content of fresh manure is around 70% to 85%.

Characteristics of animal waste depending on several of factors like: animal's breed, weight, vary eating habits and seasonal differences. Different nutrient content characterization of animal waste, are given in Table 2.4.

Ammonia emissions are known as one of the biggest environmental concern in agriculture, the main source of atmospheric NH_3 resulting from the production of animal manure and the use of inorganic fertilizers. The introduction of NH_3 and ammonium (NH_4^+) into the environment can result eutrophication and acidification effect of ecosystems. The ammonia content in manure are given in in Table 2.5.

Table 2.4: Nutrient content in different type of manure (URL-1).

	N	**P_2O_5**	**K_2O**	**Ca**	**Mg**	**Organic matter**	**Moisture content**
Fresh manure	%	%	%	%	%	%	%
Cattle	0.5	0.3	0.5	0.3	0.1	16.7	81.3
Sheep	0.9	0.5	0.8	0.2	0.3	30.7	64.8
Poultry	0.9	0.5	0.8	0.4	0.2	30.7	64.8
Horse	0.5	0.3	0.6	0.3	0.12	7.0	68.8
Swine	0.6	0.5	0.4	0.2	0.03	15.5	77.6
Treated manure	%	%	%	%	%	%	%
Cattle	2.0	1.5	2.2	2.9	0.7	69.9	7.9
Sheep	1.9	1.4	2.9	3.3	0.8	53.9	11.4
Poultry	4.5	2.7	1.4	2.9	0.6	58.6	9.2

Table 2.5: Ammonia content in manure(URL-2).

(Kg/T semi-solid)	Value
Poultry manure	4.2
Swine manure	2.4
Dairy manure	1.9
Beef manure	0.8

Mostly, three waste categories can be distinguished:

- *Liquid manure or slurry*. Housing system collecting all animal excreta in liquid form. The animal are kept on sloping solid floors that are properly sweep out, dilution can be expected from wash water;
- *Mixed manure.* Housing systems producing liquid and solid manure waste; animals are kept on bedding material, but liquids are drained from the bedding and collected in different place;
- *Solid manure.* Housing types producing only solid manure; animals are kept on bedding material which is collected together with all excreta as solid or farm yard manure (Martinez, et al., 2003).

2.8.1 Poultry manure

Like other livestock manures, poultry manure are also potential sources of many major environmental problems. Annually production of Solid waste by poultry farms has been estimated at millions of tons (Sakar, et al., 2009). Waste of poultry industry includes any kind of bedding material or litter (e.g. wood shavings or straw), a mixture of excreta (manure),waste feed, dead birds, broken eggs and feathers removed from poultry houses. Other wastes include those from cage, conveyer belt and water flushing systems (Keleher, 2001).

The chemical composition of poultry manure vary and depends of several factors such as feed of animals, source of manure, age and condition of animals, handling and storage of manure and litter used (Mariakulandai and Manickam, 1975). Poultry manure contains all the essential nutrients that are used by plants. These include potassium, calcium , magnesium, sulphur, manganese, copper, zinc , chlorine, boron, iron, molybdenum ,nitrogen and phosphorous. Poultry manure composition represent like 3-5% nitrogen, 1.5-3.5% phosphorous and 1.5-3.0% potassium and micro-nutrients at considerable amount (Amanullah et al., 2010). The amounts of these nutrients can vary depending upon many factors including the age and diet of the flock, as well as the moisture content and age of the manure.

Compared to the other manure poultry manure is difficult to handle because of high water content and semi-solid in nature. The fresh poultry manure contains 60-70%

moisture. During storage, significant amount of N is lost. Deep litter with 22% moisture, when stored in open air, rapidly loses its N due to high proteolytic activity.

In litter of meat poultry, losses up to 30% are found (Amanullah et al., 2010). Lorimor et al. (2000) stated that manure handling characteristics vary as consistency changes from liquid to solid. Solid manure normally has more than 20% solids. The more difficult handle manure, those which containing 5 to 20% solids. The moisture content of the manure is the main determining characteristic, although solids size, and the presence of bedding also can influence the equipment needed for handling, treating, and transporting. On the other hand sand is another challenging solid that is sometimes used as dairy bedding. Special settling and handling procedures requires for sand due to its high density and abrasiveness. Nutrient values are related to solid concentrations. In general, the higher the solid concentration the higher the nutrient concentration.

HIgh level of organic nitrogen a presented in poultry manure. Nitrogen exists in several forms and is constantly transformed by microbial activity, and changes in temperature, pH, moisture and oxygen concentration (Xiao Dong, 2002). The nitrogen in fresh manure, 60–80% is typically in organic form, such as urea and protein. Depending on environmental conditions a large percentage of this organic nitrogen (40–90%) is converted to ammonia within a year. NH_3 gas can be lost to the atmosphere while NH_4 can be transformed by microorganisms to nitrate (the process known as nitrification).

A portion of the nitrogen in poultry manure is in the ammonium (NH_4^+) form. Ammonium (NH_4^+) and ammonia (NH_3^-) can interchange rapidly depending on the pH. Ammonium will convert to ammonia at a pH greater than 6.5. Increasing the pH (more alkaline or less acid) increases the amount of ammonia and decreases the amount of ammonium. Most manure has a pH close to 7.0 (Amanullah et al., 2010).

The organic components of poultry litter can be classified into broad biological groups: proteins, carbohydrates and lipids or fats. Carbohydrates make up the bulk of the biodegradable material and contain cellulose, starch and sugars. The proteins are large complex organic materials composed of hundreds of thousands of amino acid groups. Lipids or fats are materials containing fatty acids (Ahmad, 2010). Some

chemical and physiochemical characterization of poultry manure are summarized in Table 2.6.

With environmental regulations becoming more stringent, regulatory compliance has become a matter of increasing concern to the poultry and livestock industries, and there is a need to install more effective waste treatment facilities. Anaerobic digestion was regarded as a source of renewable energy in the form of methane gas and has been drawing attention to the method due to its beneficial roles in poultry waste treatment.

Table 2.6 : Chemical and physiochemical characterization of solid poultry manure (Guerra-Rodriguez et al., 2001).

Parameter	Unit	Value
Organic matter content	% dry matter	85.38
pH	-	8.8
Moisture	% wet weight	48.69
Total nitrogen	% dry weight	3.56
Inorganic nitrogen	% dry weight	1.74
Ammonia nitrogen	% dry weight	1.76
OCC/nitrogen ratio	%	10.89
TCC/nitrogen ratio	%	12.24
P_2O_5	% dry weight	0.71
K_2O_5	% dry weight	3.79

2.8.2 Anaerobic treatment of chicken manure

Anaerobic digestion of manure, in particular poultry manure, is a relatively effective conversion process of litter in biogas. Biogas from poultry litter contain 60% of methane. Systems must have a certain minimum amount of poultry litter to supply and operate a given system (Kelleher, et al., 2001).

Commonly the anaerobic treatment process of poultry litter involves two distinct stages (Williams, 1999). In first stage of treatment process complex components, including fats, proteins and polysaccharides, are broken down and hydrolyzed to their component subunits. This is facilitated by facultative and anaerobic bacteria, which then subject the products of hydrolyses to fermentation and other metabolic processes leading to the production of simple organic compounds. The second stage

involves the conversion of the hydrolysis products to gases (mainly methane and CO_2) by several different species of strictly anaerobic bacteria and is referred to as methane fermentation. The two stages are illustrated in Figure 2.6.

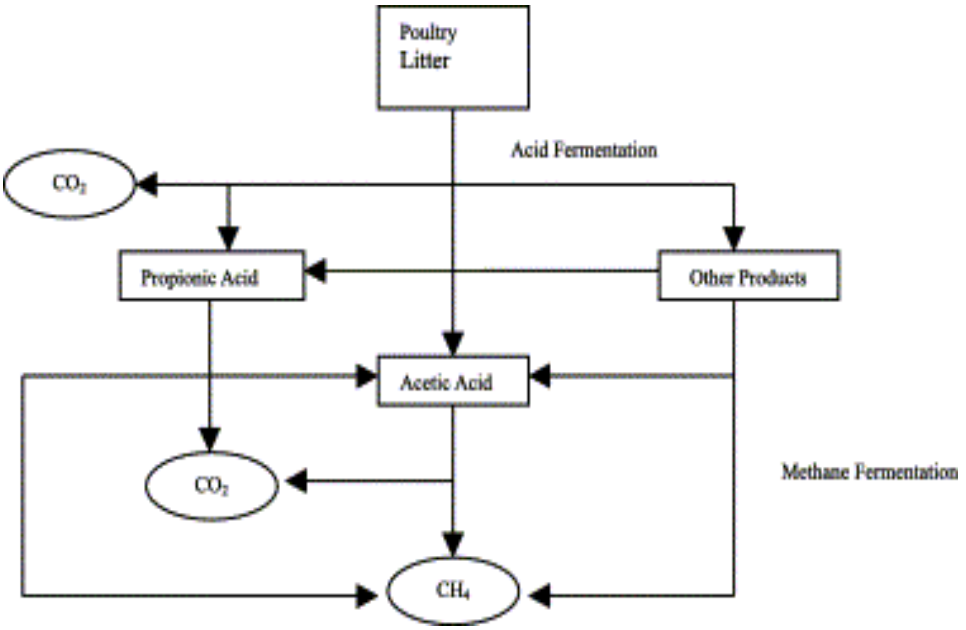

Figure 2.6 : Pathways in anaerobic digestion (Kelleher, et al., 2001).

The concentration of endogenous ammonia-nitrogen rises extensively during anaerobic digestion of poultry litter. While a certain amount of ammonium ions can be utilized by some anaerobic bacteria, an excess of ammonium can inhibit the destruction of organic compounds, production of volatile fatty acids and methanogenesis. Krylova et al. (1997) found that an excess of ammonia-nitrogen in a fermentation medium can cause inhibition process of anaerobic treatment. A possible effective solution to solve this problem is dilution of letter material to 0.5–3.0% total solids, which has the effect of eliminating ammonia inhibition.

Jones and Imre (2003) stated that anaerobic treatment does not reduce the phosphorus content in manure, and thus the liquid or sludge effluent need to be managed in a manner that handles or uses these nutrients.

During anaerobic digestion, the concentration of ammonia-nitrogen rises considerably as protein breakdown occurs. The excess of ammonium can inhibit the decomposition of organic compounds, the production of volatile fatty acids (VFAs), and methanogenesis (Xiao Dong, 2002).

2.9 Previous studies on anaerobic digestion of chicken manure

Average total COD removal efficiency in a UASB reactor inoculated with the granular sludge was reported about 95% during anaerobic treatment of diluted chicken manure at room temperature. On the other hand, average soluble COD removal efficiency in the UASB reactor was about 82%. According to TSS and VSS high removal efficiencies were are observed. Moreover, average biogas yield per gram total COD removal in the UASB reactor inoculated with the granular sludge was approximately 0.07 liter (Gulumser, 2013).

Abouelenien et al. (2010) conducted a study in which through recycling of biogas followed by gas washing in sulfuric acid ammonia was removed successfully when chicken manure was anaerobically digested for 4 days at 55 °C and at an initial pH of 8–9. By using this method, 80% of total nitrogen in chicken manure was converted to ammonia and 82% of the produced ammonia was removed. At an initial pH of 8 and at 55 °C, 195 and 157 ml $g^{-}VS^{-1}$ of methane was successfully produced from the treated chicken manure and the mixture of treated chicken manure and raw chicken manure in the ratio of 1:1, respectively. In this method, ammonia concentration was maintained at a level lower than 2 g-N kg-wet $sludge^{-1}$ in the reactor.

Bujoczek et al. (2000) performed an anaerobic digestion of high solids chicken manure in a batch screening assay. Through this study, different mixtures of the fresh manure and anaerobically digested sludge or pit manure, were incubated at 35°C. the efficiency of methane production decreased with increasing of organic loads to the digesters. The minimum solids which the digestion was still feasible was about 10% total solid. Methanogenesis took place at free ammonia (NH_3) concentrations of up to 250 mg/l. furthermore, the efficiency of organic nitrogen conversion to ammonia (NH_3+NH^{+}_4) in most digestions was ranging from 62-6% to as high as 80-3%.

Ibrahim et al. (1997) studied the performance of a UBF (upflow anaerobic sludge blanket reactor and filter material) process treatment for waste-water with chicken manure which was tested under a constant temperature of 35°C and UBF volume of 4 liters. Operated under steady state condition, the biogas production rate was 9.83 m^3/m^3 per day, at loading rate of 28.85 kg COD/m^3 per day, COD removal efficiency 80.03%, and HRT 18.73 hours.

Magbanua et al. (2001) have operated anaerobic batch tests using hog and poultry wastes in various proportions. Treatment of both wastes produced a high biogas yield (up to 200 ± 30 mL /g destroyed volatile solids (VS)) and methane yield (up to130 ± 20 mL/g destroyed VS) compared to when wastes treated alone.

Yetilmezsoy (2008), used two laboratory-scale UASB systems which were operating under different conditions for investigation of the treatment efficiency and biogas production of laying hens waste. During 140 days treatment of poultry manure in UASB systems in 1 +6 optimal solid-liquid phase mixing ratio and 12-day hydraulic retention time, an average amount of 82% COD removal and 887 L CH_4 production have been obtained. In addition, the used granular inoculum sludge (15.7 L, up approximately 30% of the volume of activated sludge) had the most important role in the successful operation of system with high performance.

Dong and Tollner (2003) studied two approaches based on new process development and biological nitrogen transformation in a bench study for investigation of removing nitrogen as N2 gas from poultry waste while stabilizing the wastes. Through this research, using serum bottles, Anammox was explored in batch anaerobic culture. The effects of ammonia levels, when addition of nitrite to poultry waste, was monitored. Results showed the 13-22% ammonium removal when inoculation with returned activated sludge. The total ammonium reduction on the other hand, was not proportional to nitrite reduction all showed that Anammox was less competitive under the examination conditions. Researchers found that the addition of nitrite and nitrate had no inhibitory effects on the biogas production and COD removal. Therefore, the classical nitrogen removal (i.e. nitrification followed by denitrification) was more effective for nitrogen removal from poultry wastes.

3. MATERIALS AND METHODS

3.1 Anaerobic sludge bed (ASB) reactor

Anaerobic treatability study was conducted in a lab-scale semi-continuous ASB reactor with an effective volume of 6.45 L Figure 3.1. The schematic view of the ASB reactor is also presented in Figure 3.2. The reactor itself consisted of a Plexiglas column of 1.0 m in height and 90 mm in diameter. A special gas-solids-liquid separator was installed on top of the reactor, and the produced biogas was collected via this separator. Five sampling ports (I, II, III, IV, and V) were installed from the bottom at 0.20, 0.36, 0.52, 0.68, and 0.84 meter heights, respectively.

Figure 3.1: The ASB reactor used in this study.

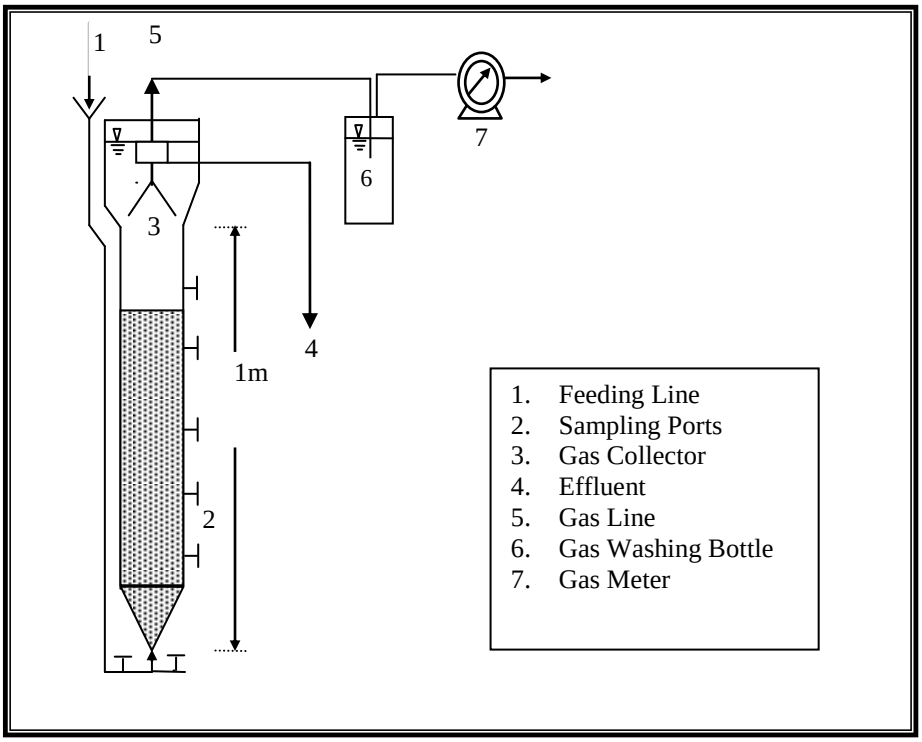

Figure 3.2: Schematic view of the ASB reactor used in this study.

3.2 Characteristic of the inoculum

The original seed was the granular sludge from the mesophilic anaerobic Internal Circulation (IC) reactor treating the wastewater produced at a pulp and paper industry with a TS concentration ca. 300 g/L (VS/TS ratio of ca. 37%). The ASB reactor was previously inoculated with ca. 0.71 L of the granular seed sludge at the start-up in order to provide the VS amount per m^3 of reactor to be in the range of 10-15 kg for an effective operation in anaerobic sludge bed reactors (Gulumser, 2013). In the scope of this study, the ASB reactor was seeded with the same active methanogenic inoculum already adapted to diluted chicken manure see Table 3.1.

Table 3.1 : Characterization of the adapted inoculum in terms of average solid concentrations.

Parameter	TSS (g/L)	VSS (g/L)	VSS (% TSS)
Value	90	46	44

3.3 Raw chicken manure

In this study, two chicken manure samples have been used taken from two different sources. The first sample was taken fresh from a family-based chicken farm with a capacity of about 50 livestocks (Manure-I); whereas the second sample was taken fresh from a big enterprise with a capacity of about 275,000 livestocks (Manure-II). Both samples were the manure from the laying-hen chicken.

Manure-I was taken fresh from a family-based chicken farm with a capacity of about 50 livestocks whereas the second source of chicken manure (Manure-II) was obtained fresh from a big enterprise with a daily capacity of about 20,000 eggs from 275,000 laying hens. Both manure samples were stored in the sealed containers at +4^{o}C. The characteristics of Manure-I and Manure-II are presented in Table 3.2.

Table 3.2 : Characterization of Manure-I and Manure-II based on average solid concentrations.

Manure-I	
TS (%)	40
VSS (% TS)	43
Moisture (%)	60
Manure-II	
TS (%)	26
VSS (% TS)	60
Moisture (%)	74

3.4 Diluted chicken manure

The substrate used in this study was the diluted raw laying-hen manure. In this scope, Manure-I and Manure-II were both diluted before fed into the ASB reactor with an appropriate influent feed ratio (kg of fresh chicken manure to liter of tap water) of 1 to 6 (1kg manure to 6L tap water). Waste slurry of Manure-I (Slurry-I) and Manure-II (Slurry-II) were also kept at +4ºC. Characterization of both influent waste slurries used in this study is presented in Table 3.3.

Table 3.3 : Characterization of Slurry-I and Slurry-II (1 kg chicken manure + 6 L tap water) used in this study.

Parameters	Unit	Mean ± Std. Dev.	Median
		Slurry-I	
Total COD	mg/L	27022±7671	27630
Soluble COD	mg/L	4712±2384	4108
TSS	mg/L	48200±23080	45247
VSS	mg/L	26596±6624	24688
Alkalinity (as $CaCO_3$)	mg/L	3650±733	3300
pH	-	7.82±0.30	7.72
		Slurry-II	
Total COD	mg/L	28123±6152	29635
Soluble COD	mg/L	10498±2852	11916
TSS	mg/L	36947±19655	28508
VSS	mg/L	22530±13173	17404
Alkalinity (as $CaCO_3$)	mg/L	3285±896	2900
pH	-	7.62±0.34	7.59

3.5 Operating conditions at the ASB reactor

The start-up period for the ASB reactor was already completed as described in Gulumser (2013).Thus, the granular inoculum has been already adapted to the diluted chicken manure. In the scope of this study, the ASB reactor was continued to be fed upwards with the diluted chicken manure (with an influent feed ratio of 1kg manure to 6L of tap water). The ASB reactor fed with Slurry-I was operated at HRT of ca. 8.6-13 day (Qslurry=500-750 mL/day) for about 3 months; whereas the ASB reactor fed with Slurry-II was operated at HRT of ca. 13-26 day (Qslurry=250-500 mL/day), for about 6 months

3.6 Analytical methods

Total COD, soluble COD, total solids (TS), total volatile solids (VS), total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_3-N), total phosphorus (TP) and alkalinity parameters were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). Total COD and soluble COD analyses were performed by the dichromate open reflux titrimetric method. The samples were filtered through Cellulose Nitrate Filters having pore sizes of 0.45 μm for soluble COD analyses. For TSS and VSS analyses of the influent samples, fresh samples were taken and were centrifuged by the apparatus Hettich Zentrifugen Universal 320 model for 15 minutes at 9000 rpm for the separation of soluble and suspended solids. The pellets were used for TSS and VSS analyses. The pellets after centrifugation were transferred into crucibles and dried overnight at 105°C for TSS determinations. On the other hand, TSS and VSS concentrations of the effluent were conducted according to gravimetric method. In this scope, the effluent samples were filtered through AP40 filters and dried for 1 hour at 105°C for TSS and burned for 30 min for VSS concentrations.

For the solids concentration of the raw manure samples, the tared crucibles were first dried on the water bath and then dried overnight at 105°C for TS determination. VS concentration of the raw manure samples was determined by ignition at the oven (550°C) for 30 minutes. Ammonium nitrogen was measured using distillation-titration method. Samples were first buffered with borate buffer solution at pH=9,5 and distilled into boric acid solution before titrated with 0,02 N sulphuric acid. The pH values in the samples were measured by HI 2211-02 HANNA Model pH meter. Temperature and the biogas produced were measured daily. Total biogas was measured by Ritter Milligas Counter 770991000 Model gas meter (Figure 3.3). Its methane and carbon dioxide content were measured by were measured by Perichrom PR2100 Model GC with TCD detector.

Anaerobic treatment performance of the ASB reactor was evaluated by measuring the parameters of taken samples from the inlet and outlet of the reactor and showed in Table 3.4.

Figure 3.3 : The gas meters used in this study.

Table 3.4 : The analysis performed in this study and the measurement frequency**.**

Parameters	Frequency of Analysis
Total COD	3 / Week
Soluble COD	3 / Week
NH_3-N	2 / Month
TSS and VSS	3 / Week
Alkalinity	3 / Week
pH	3 / Week
Temperature	Daily
Biogas	Daily

During the study, the samples of the inlet and the outlet of the reactor were taken for the analysis and all results were calculated using the Microsoft ® Excel (2007) program.

3.7 Microbiological studies

3.7.1 Genomic DNA (GDNA) extraction

Genomic DNAs were extracted from 1-ml sludge sample using FastDNA Spin Kit for Soil (Qbiogene Inc., U.K.) following the manufacturer's instructions. Extracted GDNA concentration was determined Nanodrop 2000 (Thermo Scientific, USA) and diluted to 25ng/ml by DNase free water.

3.7.2 Quantitative real time polymerase chain reaction (Q-PCR)

3 primer sets targeting the bacteria, archaea and methanogens were used to quantify the existing microbial community by using the template extracted GDNAs. All primers used for Q-PCR analysis are given in Table 3.5.

Table 3.5 : Q-PCR primers used in the study.

Primer Sets	Target	Annealing	Reference
Bac519-Bac907r	16rDNA	53	Lane,1991
Arc-344f Arc-855r	16rDNA	60	Tkai, 2000
Met348f Met 786r	16rDNA	55	Sawayama,2006

The procedure recommended by Roche was followed and Light Cycler Master Kit (Roche, Applied Science, Switzerland) was used to set up the reaction (2.0 ml master mix, 1.6 ml $MgCl_2$ 1.0 ml Primer F and R, 13.4 ml H_2O, 1 ml sample). Absolute quantification analysis of the GDNA was carried out with a LighCycler 480 Instrument (Roche, Applied Science, Switzerland). The amplification protocol was as follows: initial denaturation for 10 min at 94 $^{\circ}$C followed by 45 cycles of 10 s at 94°C, 5 s at specific annealing temperature 16s at 72°C. The standard curves for Q-PCR.

For each PCR run with SYBR Green I detection, a melting curve analysis was performed to confirm the specificity in each reaction tube by the absence of primer dimers and other nonspecific products. Reactions for all samples were shown to have only melting peak, which indicated a specific amplification making it suitable for accurate quantification.

4 RESULTS AND DISCUSSION

The 'Results and Discussion' part contains the results and discussion which will be presented as the first and the second sections for Slurry-I (from 30th Mart to 12th July 2013) and Slurry-II (15th July 2013 to 10th January 2014), respectively. In this scope, two different manure slurries were conducted in this study as described in the Materials and Methods part. The procedures were the same during the whole experimental period with both slurries unless where stated otherwise.

4.1 Anaerobic treatability of Slurry-I

At the first step, SMA (specific methanogenic activity) test was conducted in order to characterize the granular inoculum (i.e. that has already been adapted to chicken manure) in terms of acetoclastic methanogenic activity. For this purpose, acetate concentrations in the range of 1000-5000 mg/L were tested in order to obtain the maximum potential methane production (PMP) rate. SMA results indicated that maximum PMP rate was obtained for the acetate concentration of 4000 mg/L with a methane production of about 140 ml CH_4/g VSS. It was concluded that the seed sludge had high potential and it was suitable to be used as the inoculum in the digestion process.

4.1.1 Total COD and soluble COD changes

COD removal efficiency determines performance of the anaerobic digestion process. It is expected from any anaerobic treatment or biodegradation process for COD to be reduced. In this experiment, it was found that there was an efficient removal of COD (HRT≅24 hr.). Total and soluble COD concentrations of the influent were measured as 28546±9662 mg/L and 4788±2497 mg/L, respectively (Table 4.1). Total COD removal efficiency through the study was almost consistent and total and soluble COD removals were calculated about 89±5% and 63±14%, respectively (OLR=2.2-3.3 kg COD/m^3.day) (Figures 4.1 and 4.2).

Table 4.1 : Total COD and Soluble COD changes in the ASB reactor treating diluted chicken waste**.**

Parameters	Unit	Minimum	Maximum	Mean ± Std. Dev.	Median
Total COD $_{influent}$	mg/L	8727	57283	28546±9662	27630
Total COD $_{effluent}$	mg/L	427	4246	2696±804	2985
Total COD removal	%	74	98	89±5	89
Soluble COD $_{influent}$	mg/L	2218	11942	4788±2497	4108
Soluble COD $_{effluent}$	mg/L	397	2.368	1483±361	1503
SolubleCOD removal	%	29	88	63±14	64

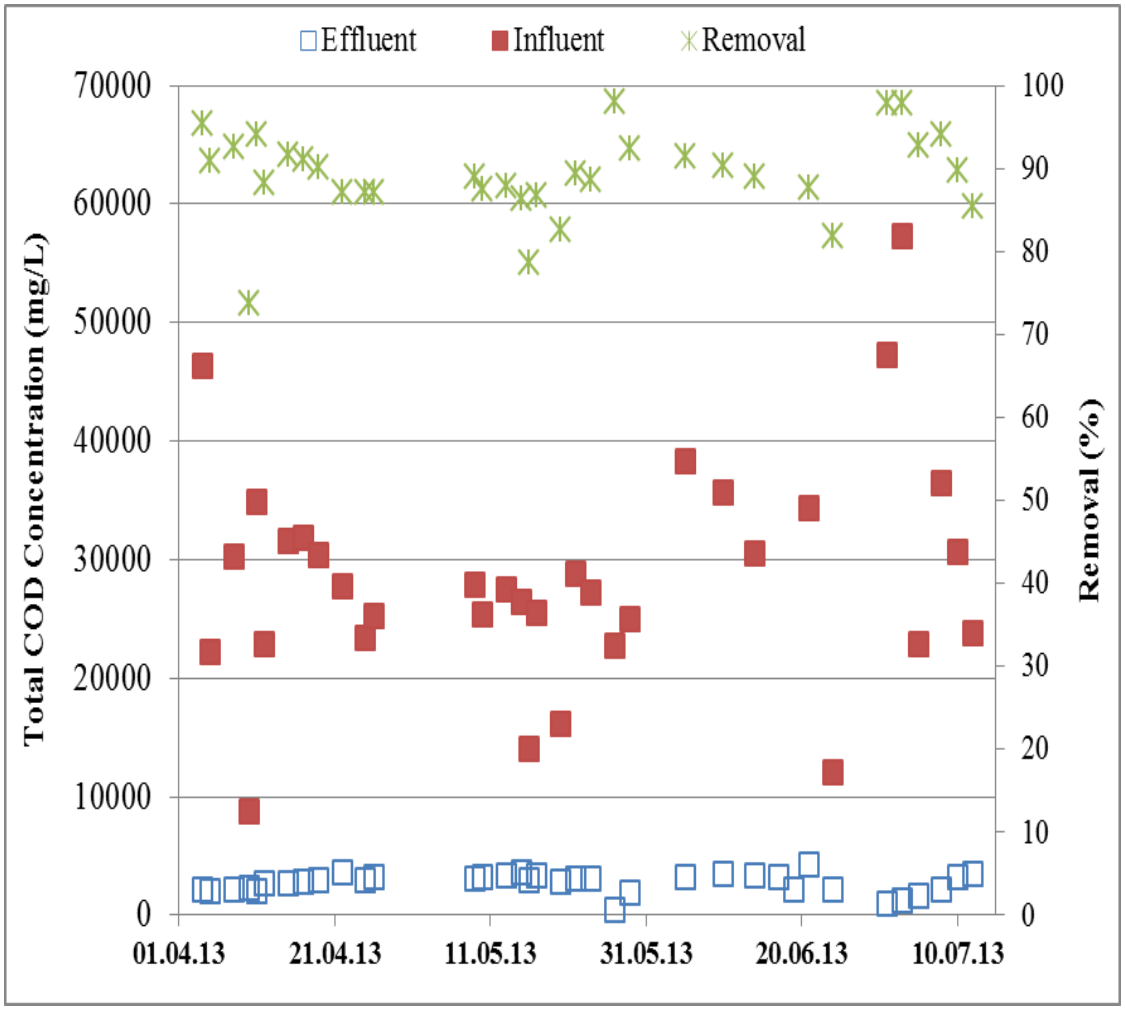

Figure 4.1 : Total COD changes in the ASB reactor treating Slurry-I.

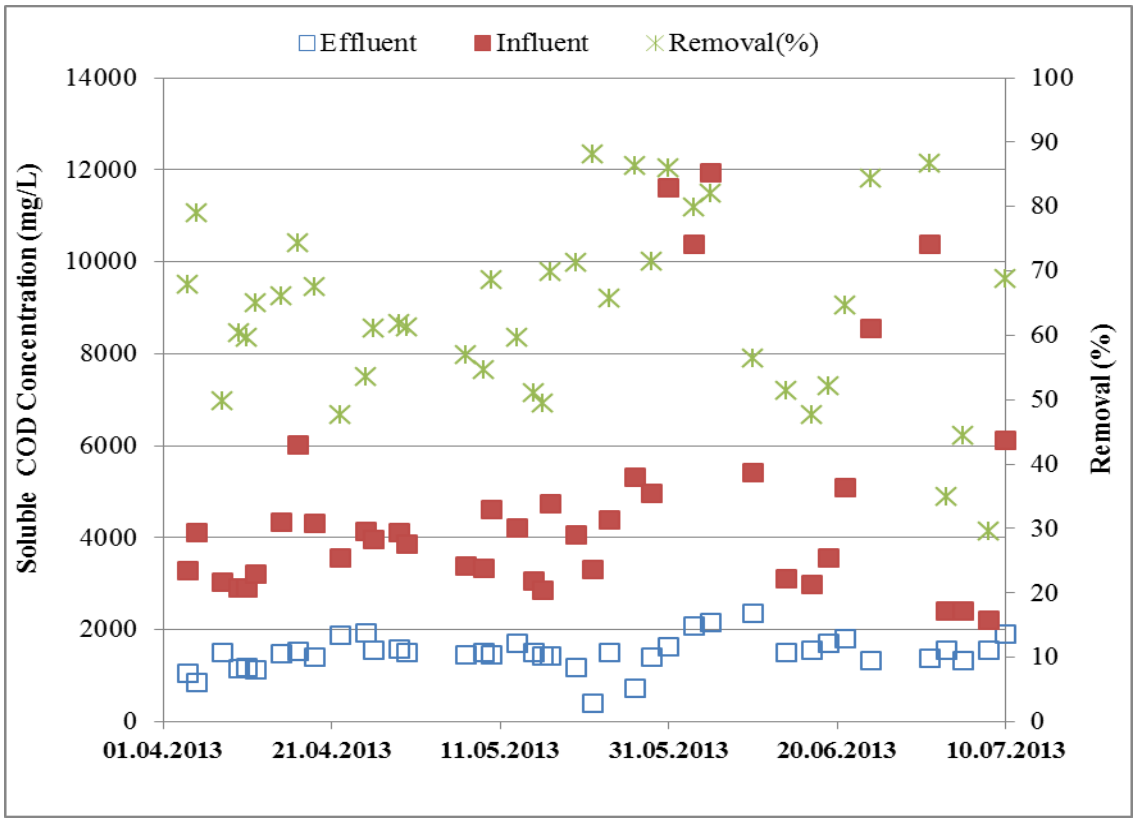

Figure 4.2 : Soluble COD changes in the ASB reactor treating Slurry-I.

4.1.2 TSS and VSS changes

Results indicated that influent and effluent TSS concentrations were observed as 50951±23124 mg/L and 772±313mg/L, respectively. Although significant fluctuations were observed in the influent waste slurry, they indicated stability in the effluent of the reactor (Figures 4.3 and 4.4). Results also indicated high removal rates in terms of suspended solids that showed stability during the study. Influent and effluent TSS and VSS concentrations as well as their removals are given in Table 4.2.

Table 4.2 : TSS and VSS changes in the ASB reactor treating diluted chicken waste**.**

Parameters	Unit	Minimum	Maximum	Mean ± Std. Dev.	Median
TSS $_{influent}$	mg/L	8440	127626	50951±23124	46016
TSS $_{effluent}$	mg/L	77	1855	772±313	751
TSS removal	%	88	99	98±182	98
VSS $_{influent}$	mg/L	9916	56111	27739±9948	24975
VSS $_{effluent}$	mg/L	85	1230	555±199	541
VSS removal	%	92	99	97±1.06	98

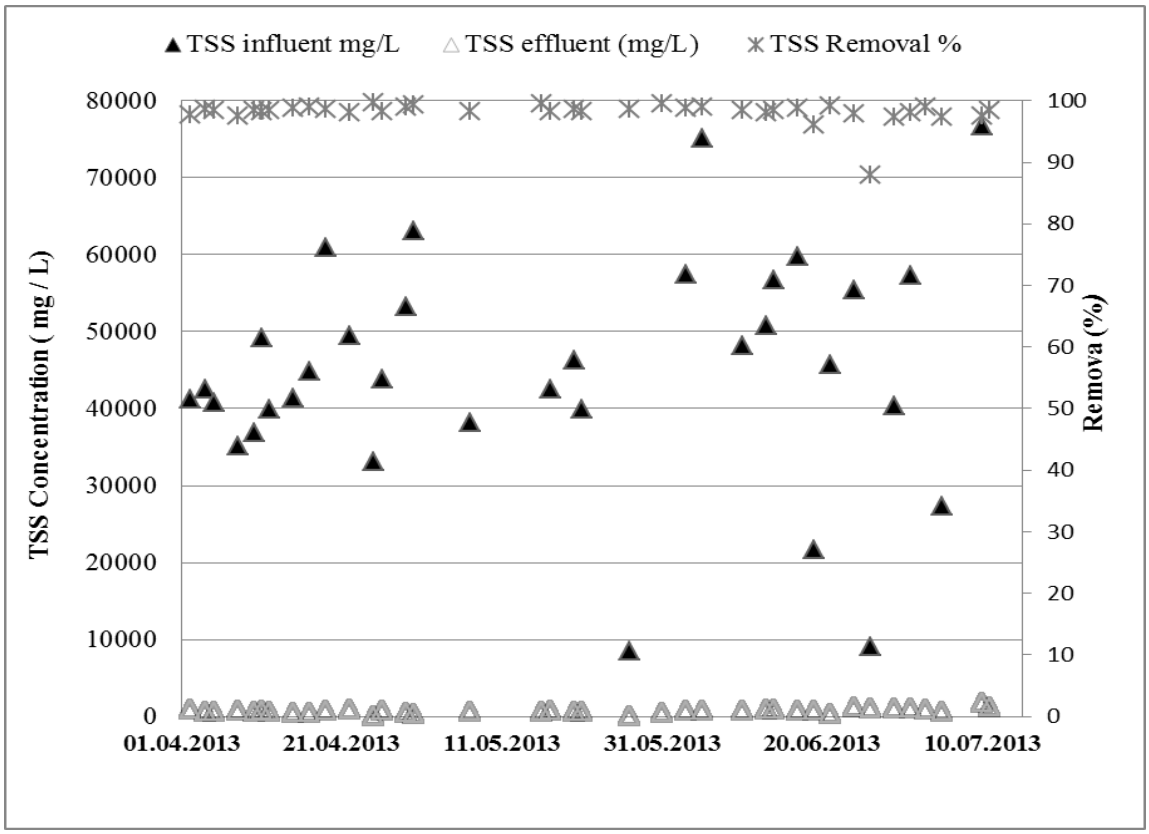

Figure 4.3 : TSS changes in the ASB reactor treating Slurry-I.

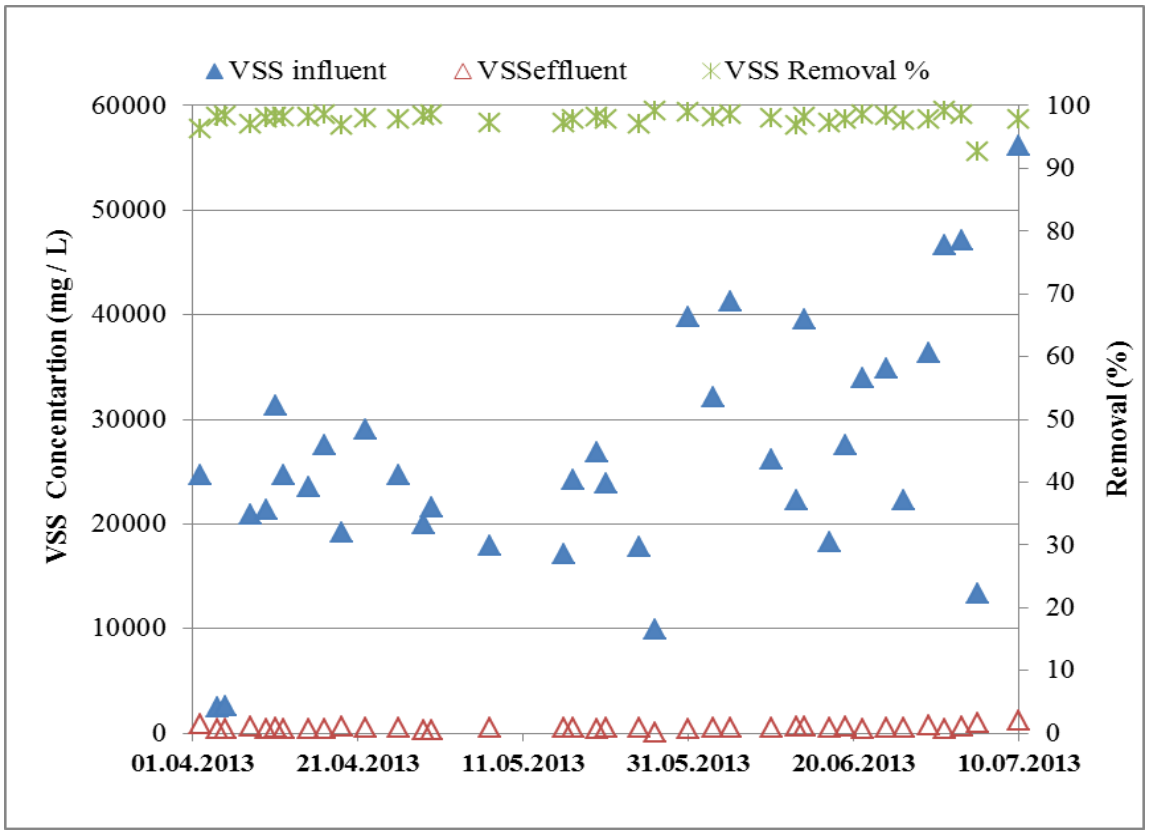

Figure 4.4 : VSS changes in the ASB reactor treating Slurry-I.

4.1.3 pH, alkalinity, and nitrogen changes

Alkalinity and pH results in the influent and effluent were ca. 3196±635 and 2763±449 mg $CaCO_3$/L and 7.82±0.30 and 8.14±0.25, respectively (Table 4.3). pH and alkalinity changes in the influent and effluent of the reactor are also presented in Figures 4.5 and Figure 4.6, respectively.

Table 4.3 : pH and alkalinity changes in the ASB reactor**.**

Parameters	Unit	Minimum	Maximum	Mean ± Std. Dev.	Median
$pH_{influent}$	-	7.75	8.56	7.82±0.30	7.72
$pH_{effluent}$	-	7.67	8.47	8.14±0.25	8.20
$Alkalinity_{influent}$	mg $CaCO_3$/L	1970	4600	3196±635	3300
$Alkalinity_{effluent}$	mg $CaCO_3$/L	1840	3600	2763±449	2750

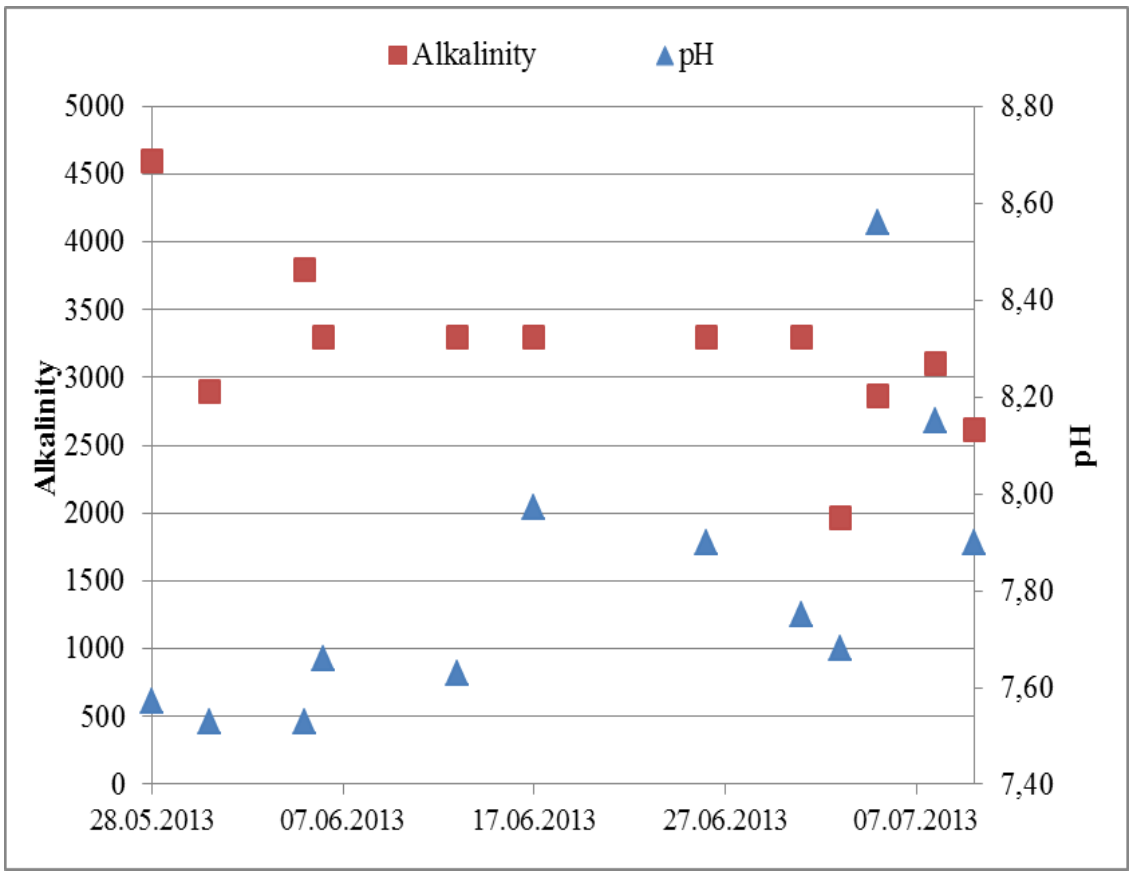

Figure 4.5 : pH and alkalinity changes in the influent in the ASB reactor treating Slurry-I.

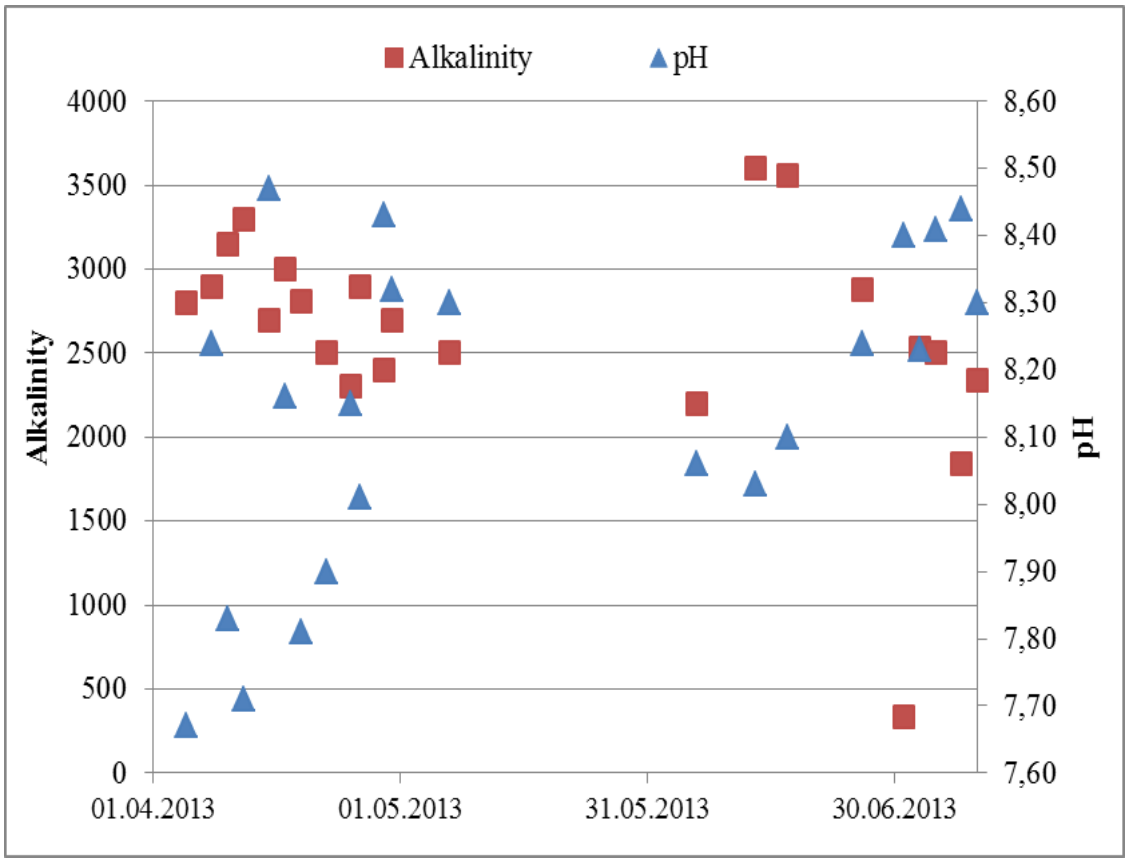

Figure 4.6 : pH and alkalinity changes in the effluent in the ASB reactor treating Slurry-I.

Ammonia nitrogen concentrations in the influent and in the effluent were 323 and 817 mg/L, respectively. As expected from all anaerobic systems, NH_3-N generally increases when subjected to anaerobic treatment. It is reported that total NH_3-N concentrations may be up to 4000 mg-N/L especially when digesting raw poultry manure due to high ammonia contents in this waste. Hence, digestion of the poultry manure without dilution has previously been shown to be unsuccessful. Results of this study showed that although NH_3-N concentration inside the ASB reactor was measured above 800 mg/L, performance of the reactor did not indicate any free ammonia inhibition in terms of biogas production. Thus, tolerance to high total NH_3-N has been demonstrated by adaptation of the reactor to ammonia in the diluted chicken waste. Since, the free ammonia has been reported to be the active component causing inhibition at high pH levels, the pH in the ASB reactor was controlled constantly and the pH results were not measured above 8.2 during the study (Yangin-Gomec and Ozturk, 2013; Angelidaki and Ahring,1993).

The operational temperature of digesters is essential for the stable treatment of anaerobic system. Temperature factor plays an important role in both thermodynamics and kinetics of the reactions which intermediate by some microbes. At the same time, chemical equilibriums are also affected by the operating temperature, especially for the concentration of free ammonia at a fixed total ammonium concentration. At higher temperatures the ratio of free ammonia to the total ammonium will be higher. When free ammonia is inhibiting to methanogenesis, higher temperatures can inhibit methane generation in anaerobic digesters. And because of this reason, ammonium-, urea-, and protein-rich animal wastewaters are difficult to treat under thermophilic conditions (55–65 °C) and even though the kinetics are favorable compared to mesophilic conditions (25–37 °C) (Angenent and Garcia, 2009).

Investigating inhibitory effects of ammonia nitrogen on anaerobic treatment process should be carried out by controlling important parameters such as pH, temperature, retention time and organic loading rate (Calli, 2004). The dissociation constant for NH_4^+/NH_3 depends on the temperature. An increase of temperature at constant total ammonia concentration can be the reason to an increase of free NH_3 concentration. Most authors confirmed that thermophilic micro-organisms were more active at high free NH_3 concentration than mesophilic micro-organisms. Gallert et al., 1998 found 50% of methanogenic inhibition with 88 mg NH_3-N/L at 37°C and 297 mg NH_3-N/L at 55°C, respectively. In literature sources, usually severe or complete inhibitions were reported at similar or lower free ammonia nitrogen concentrations and most of these studies were conducted under thermophilic conditions. But, methanogenic activities were reported at free ammonia nitrogen concentration as high as 1100 mg/L in thermophilic digestion of different animal manures (Calli, 2004).

It was reported that anaerobic digester effluents have other agronomic advantages because the pH in manure fed digesters increases from 7.0 to 8.0 during anaerobic digestion (Massé et al., 2011).

Considering the dual benefits of environmental pollution control and meeting national energy needs, anaerobic digestion of poultry manure wastewater has been proposed as an attractive treatment option in recent years. However, the necessity to comply with discharge limits has become a matter of increasing concern to the

poultry industry. Hence, pollutant loads discharged from poultry farms should be first reduced to a certain extent, and then an effective post-treatment unit should be installed for the anaerobically pretreated poultry manure wastewater to provide the requirements of environmental protection laws (Yetilmezsoy and Sakar, 2008).

4.2 Biogas production

Biogas from anaerobic bioreactors is an excellent substitute to fossil fuel energy sources such as coal, oil and natural gas for electricity generation and heating. In this study, daily biogas productions indicated significant fluctuations at the ASB reactor (Figure 4.7). Daily biogas generations were measured in the range of 539 - 6151 mL/day with an average biogas production of ca. 3059±1203 mL/day. Results indicated cumulative biogas generations of ca. 115 L corresponding to ca. 70 L biomethane. CH_4 content of the biogas was measured and found as about 60% during the study. On the other hand, average biogas production yield per kg of total COD removal was calculated as about 0.21 m^3 (Figure 4.8). It was reported that CH_4 is the main GHG emitted from animal slurry storage facilities, that it is highly variable. It can be substantial depending on some factors such as manure type, manure composition, manure bedding content, storage temperature, storage duration, and the formation of a natural cover (i.e., crust) at the surface of the stored manure. Anaerobic digestion has the potential to substantially eliminate uncontrolled fugitive CH_4 emissions from the stored manure, but three conditions must be met being: (1) a well-designed gas tight bioreactor which eliminates fugitive CH_4 emissions from the full scale bioreactor, (2) bioreactor design and operation must provide enough retention time to extract most of the energy from the organic substrates and, (3) the long term storage tank receiving the bioreactor effluent should have a gas tight cover to collect and recycle residual CH_4 (Massé et al., 2011).

Table 4.4 : Daily biogas production in the ASB reactor.

Parameters	Unit	Minimum	Maximum	Mean ± Std. Dev.	Median
Daily biogas	mL/day	539	6151	3059±1203	2831

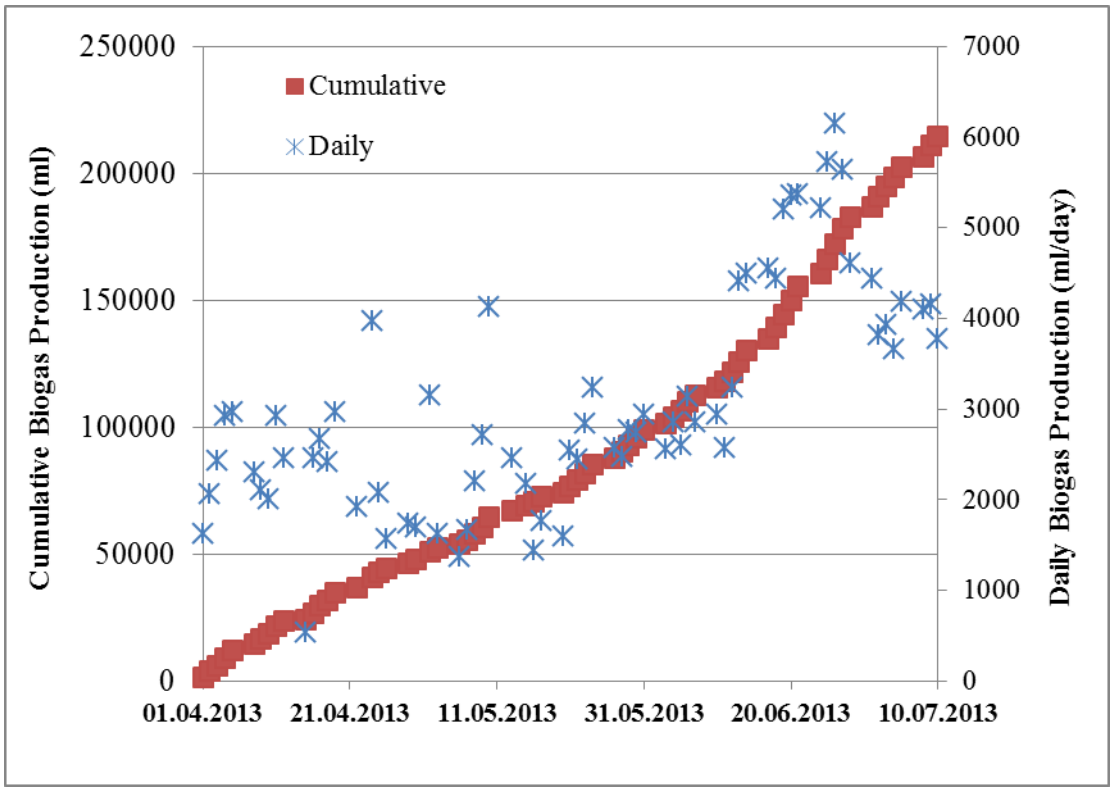

Figure 4.7 : Daily and cumulative biogas productions in the ASB reactor treating Slurry-I.

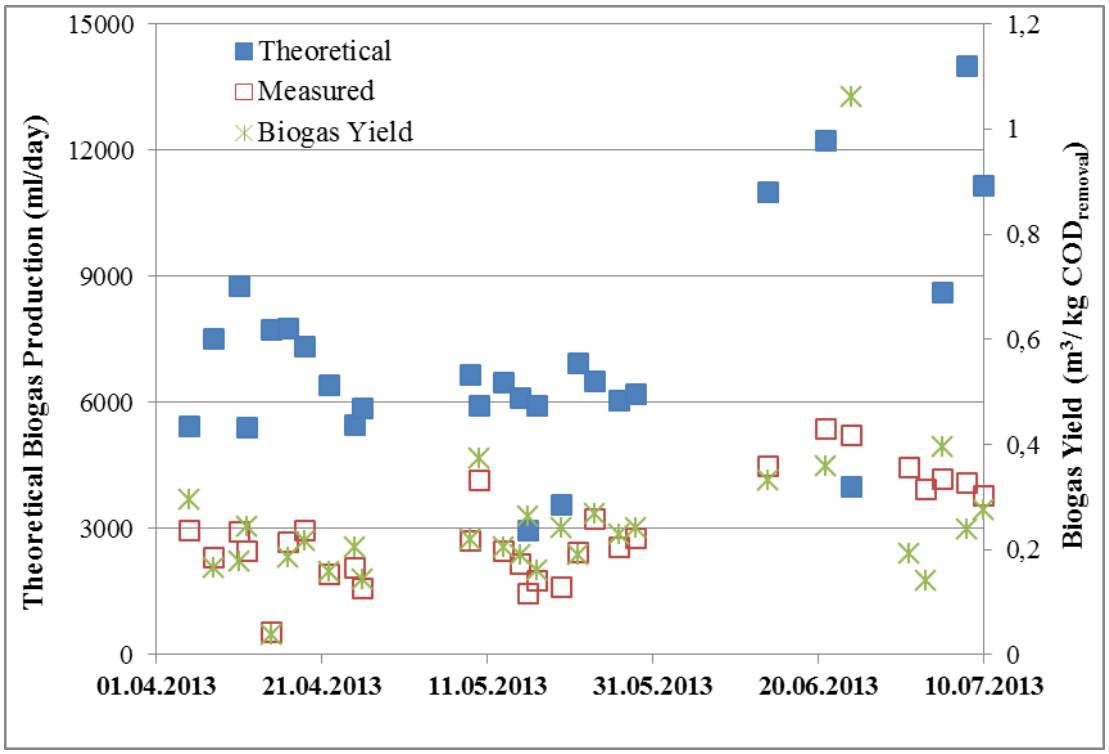

Figure 4.8 : Biogas production and biogas yield per kg total COD removal in the ASB reactor treating Slurry-I.

3.2.4 Biomass and VFA profiles along the ASB reactor

Five sludge sampling ports were installed at various heights in order to; (i) evaluate the changes in the biomass and VFA during the operation period, and (ii) to figure out the sludge distribution along the ASB reactor. Ports I, II, III, IV, and V were placed at the 0.20, 0.36, 0.52, 0.68, and 0.84 m heights from the bottom port where the feeding took place, respectively. Average sludge samples along the ASB reactor would also help to evaluate the change in the solids concentrations of the available sludge in the ASB reactor (i.e. unavoidable sludge wash-out or sludge accumulation in the sludge blanket) during the operational period. During the study, no sludge was withdrawn from the reactors. High sludge retention times (SRT) (~100 d) are often reported for the high-rate anaerobic systems such as sludge bed reactors (Cao and Ang, 2009).

Results in terms of the suspended solids concentration in the biomass samples taken at two different dates were presented in Figure 4.9 a-b. The average TSS and VSS concentrations along the ASB reactor for two sampling dates (March 29, 2013 and June 12, 2013) were 89 and 46 and 121 and 51 g/L, respectively. For the samples taken in March 29, 2013, the highest solids concentration was observed at the fourth port with a VSS/TSS ratio of about 51% in the ASB reactor. On the other hand; Ports I, II, and III also indicated high concentrations of TSS and VSS with the values all above 100 and 50 g/L, respectively. When compared with the volatile content of the original granular seed (37%), slight increase in the volatile content was observed. Hence, the high amounts of organic particles in the influent diluted chicken manure might be kept within the sludge bed. Results showed about the same volatile contents in the sludge samples from the Ports I, II, III, and IV in the range of 50-55%. However, no significant TSS and VSS were observed at the highest sampling point of the reactor (Port V) which could be attributed to the fact that sludge could be well kept inside the column where the effective digestion volume took place (Figure 4.9 a). For the samples taken in June 12, 2013, the highest solids concentration was observed at the second port with a VSS/TSS ratio of about 42% in the ASB reactor. On the other hand, significant TSS and VSS were observed at the highest sampling point of the reactor (Port V) (Figure 4.9b). Hence, it was concluded that the biomass inside the reactor was distributed along the effective digestion volume of the column.

Volatile fatty acids (VFA) were also measured from five sampling ports along the ASB reactor. The VFAs produced were mainly acetate (i.e. acetic acid was detected as more than 78% of total acid). The average total acid concentrations along the ASB reactor for three sampling dates were 100, 55, and 145 mg/L respectively. Propionic acid was detected at all sampling ports whereas valeric, isovaleric, butyric, and isobutyric acids were also found at some extent. On the other hand, isocaproic, heptanoic, and formic acids were not detected (Table 4.5). For the samples taken in March 29, 2013, the highest total acid concentration was at the fifth port with about 84% and 16% of acetic and propionic acids, respectively. On the other hand, Port II indicated the lowest concentrations in terms of total acid and acetate. For the samples taken in June 12, 2013, the highest total acid concentration was at the second port with about 86% of acetic acid and total acid was measured as 59±12 mg/L in the samples taken from Ports I, III, IV, and V (Table 4.5).

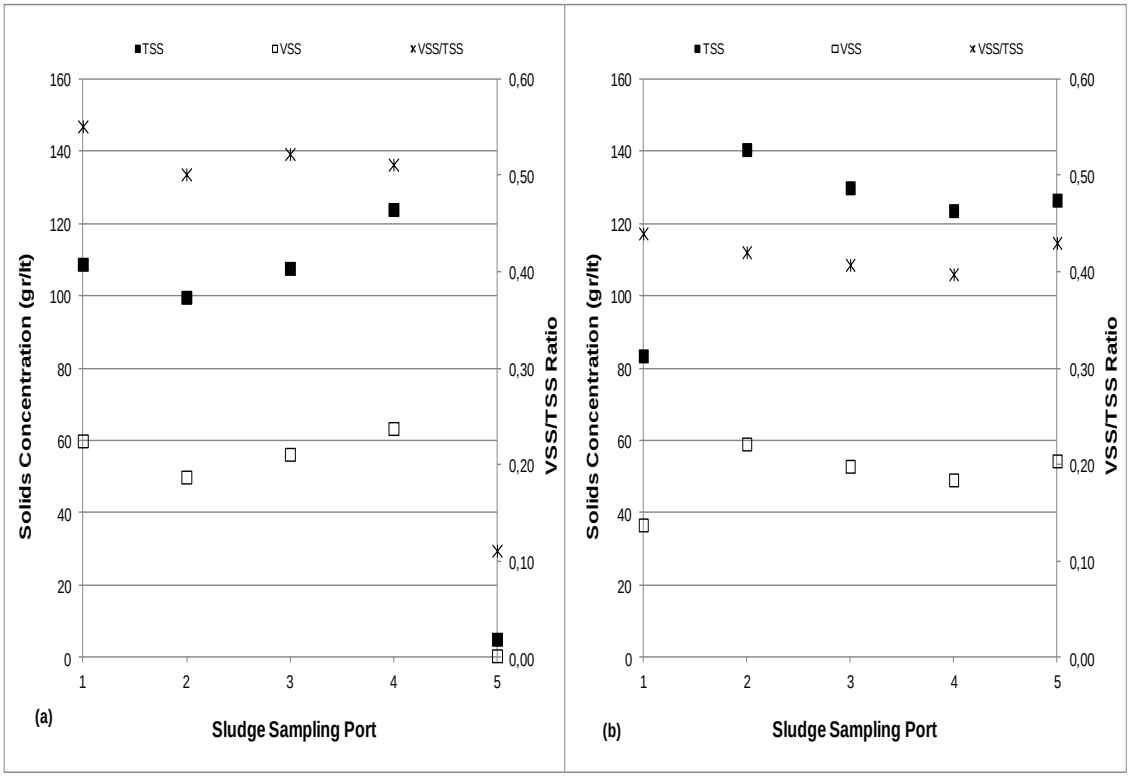

Figure 4.9 : Changes in the solids along the ASB reactor for sampling dates; (a) March 29, 2013 (b) June 12, 2013.

Table 4.5 : Changes in the VFA concentrations (mg/L) along the ASB reactor for different sampling times.

Port	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Heptanoic	Formic	Total
Sampling Date: March 29, 2013										
1	35	17	0	0	0	0	0	0	0	51
2	29	13	0	0	0	0	0	0	0	42
3	112	21	0	0	0	0	0	0	0	132
4	109	20	0	0	0	0	0	0	0	130
5	121	23	0	0	0	0	0	0	0	143
Sampling Date: May 08, 2013										
1	34	0	6	0	0	0	0	0	0	39
2	42	5	7	0	0	0	0	0	0	54
3	30	8	0	0	0	0	0	0	0	39
4	56	6	13	0	0	0	0	0	0	75
5	54	9	5	0	0	0	0	0	0	68
Sampling Date: June 12, 2013										
1	41	0	5	0	0	0	0	0	0	47
2	421	15	16	12	21	4	0	0	0	489
3	46	0	6	0	0	0	0	0	0	52
4	56	6	8	0	0	0	0	0	0	70
5	55	5	8	0	0	0	0	0	0	68
Effluent	47	0	0	0	0	0	0	0	0	47

Anaerobic digestion of organic matter in livestock manures is a natural mineralization process completed by microbial consortia composed of hydrolytic and fermentative bacteria as well as acetogens and methanogens. In anaerobic digestion: (1) hydrolysis of solid organic particles and high molecular weight compounds such as polymers which are too large to permeate the cell membrane into soluble and metabolizable molecules small enough to diffuse across the membrane, (2) the sugars, lipids and amino acids resulting from carbohydrate and protein hydrolysis are transformed into VFA, H_2 and CO_2 by fermentative bacteria, (3) H_2 producing acetogens oxidize VFA with more than two C, and long chain fatty acids resulting from lipid hydrolysis, into acetic acid, H_2 and CO_2. These bacteria work in synchrony with methanogens which consume the H_2 produced during oxidation, (4) homoacetogenic bacteria transform CO_2 and H_2 into acetate, (5) acetoclastic methanogens degrade acetic acid into CH_4 and CO_2, (6) H_2 utilizing hydrogenotrophs (i.e., methanogens) reduce CO_2 to CH_4 (Massé et al., 2011).

3.2.5 Temperature

The operating temperature inside the ASB reactor was controlled and recorded daily during the study. Results indicated an average operational temperature as 21°C. Results indicated that the ASB reactor has been operated at sub-mesophilic conditions due to the fact that the temperature values inside the reactor were measured in the range of 17-25°C. However, presently, nearly all treatment systems are operated under mesophilic temperature conditions (>18°C), whereby a

considerable amount of energy is required to heat the bioreactors at treatment temperature (Lettinga et al., 2001). Thereby, effective operation of bioreactors under ambient temperature or low-temperature (<18 °C) would decrease the treatment costs of waste streams at sub-mesophilic temperatures, creating low temperature anaerobic digestion system an attractive option for the treatment of a variety of waste categories. Low temperature anaerobic digestion, at laboratory-scale, based on the expanded granular sludge bed bioreactor design, demonstrated as an effective treatment option for a number of wastewaters (O'Flaherty et al., 2009). The change in the temperature during the operational period is given in Figure 4.10.

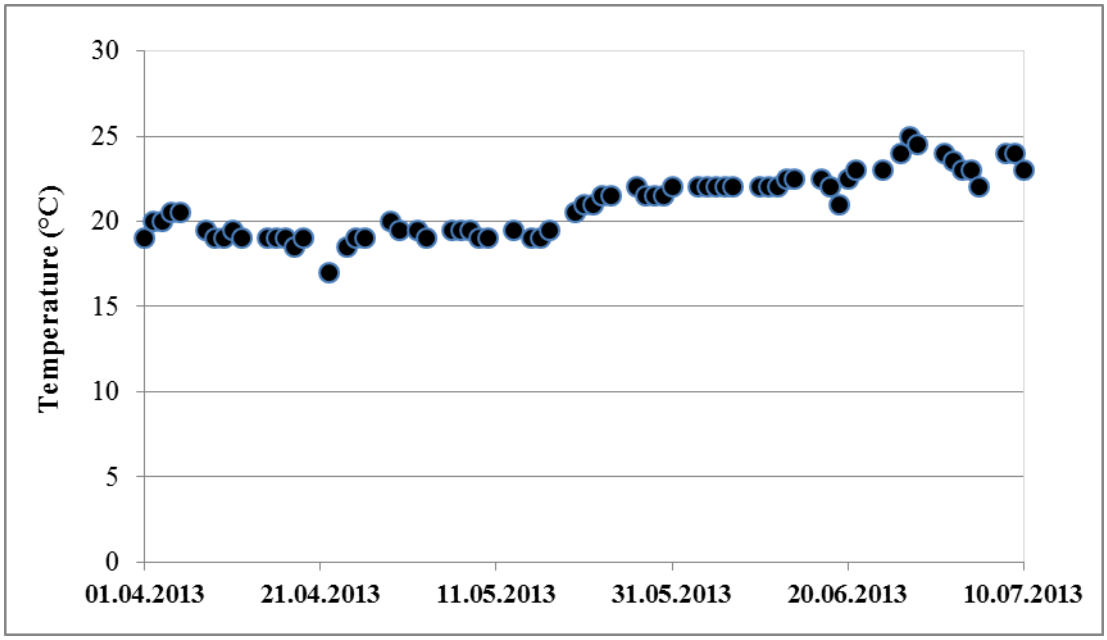

Figure 4.10 : Operating temperature changes inside the reactor treating Slurry-I.

3.3 Anaerobic Treatability of Slurry-II

Slurry-II was started from the 98th day of reactor operation and like in Slurry-I, the same parameters were monitored and controlled. Being a new waste, some of the results might differ from the results of the study with Slurry-I.

3.3.1 Total COD and soluble COD changes

Results indicated an average total COD and soluble COD removals of around 90±5% and 75±7%, respectively (OLR ~ 2.0 kg COD/m^3.day). Changes of total and soluble COD during the study are shown in Figures 4.11 and 4.12, respectively. Results also indicated that average soluble COD removal efficiency of Slurry-II was better than

that of Slurry-I. Moreover, results showed significant fluctuations in terms of COD concentrations in the influent of the ASB reactor. However, relatively less fluctuation in the effluent of the reactor has been observed. The changes in COD parameters are presented in Table 4.6.

Table 4.6 : Total COD and Soluble COD changes in the ASB reactor treating diluted chicken waste.

Parameters	Unit	Minimum	Maximum	Mean ± Std. Dev.	Median
Total COD $_{influent}$	mg/L	9258	43723	28344±6758	29449
Total COD $_{effluent}$	mg/L	1740	3807	2723±557	2797
Total COD removal	%	66	94	90±5	90
Soluble COD $_{influent}$	mg/L	3607	16613	10510±2742	10931
Soluble COD $_{effluent}$	mg/L	893	3.391	2442±751	2743
Soluble COD removal	%	64	93	75±7	74

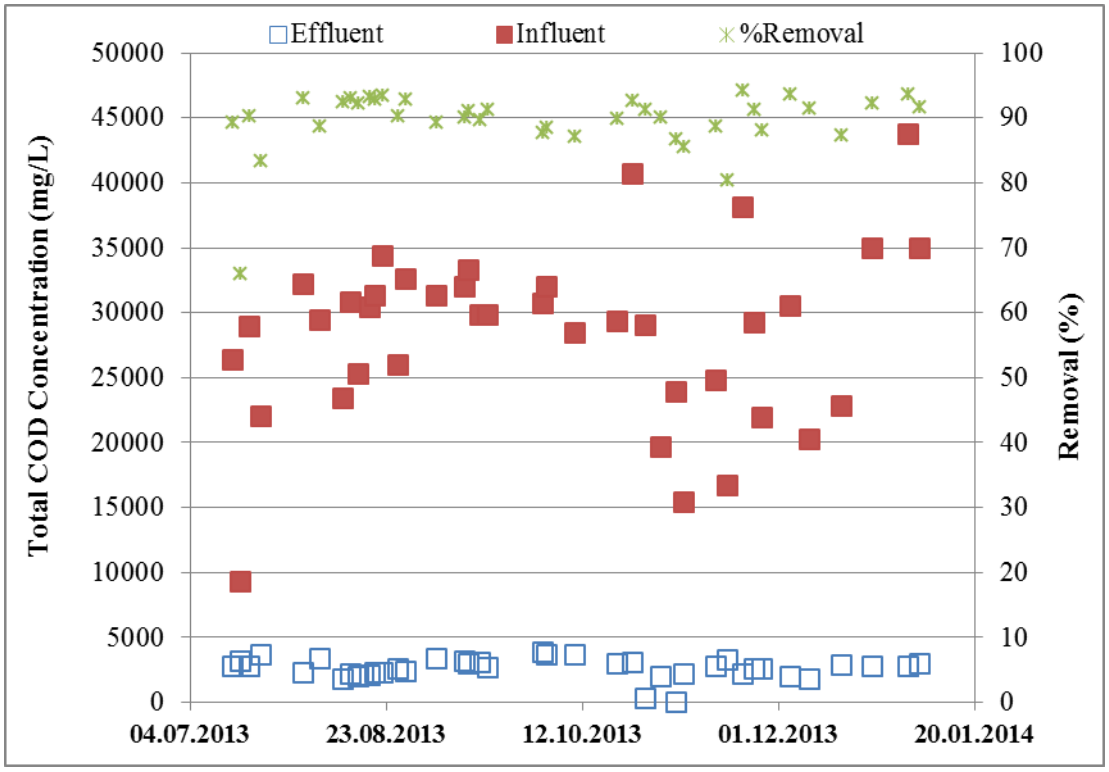

Figure 4.11 : Total COD changes in the ASB reactor treating Slurry-II.

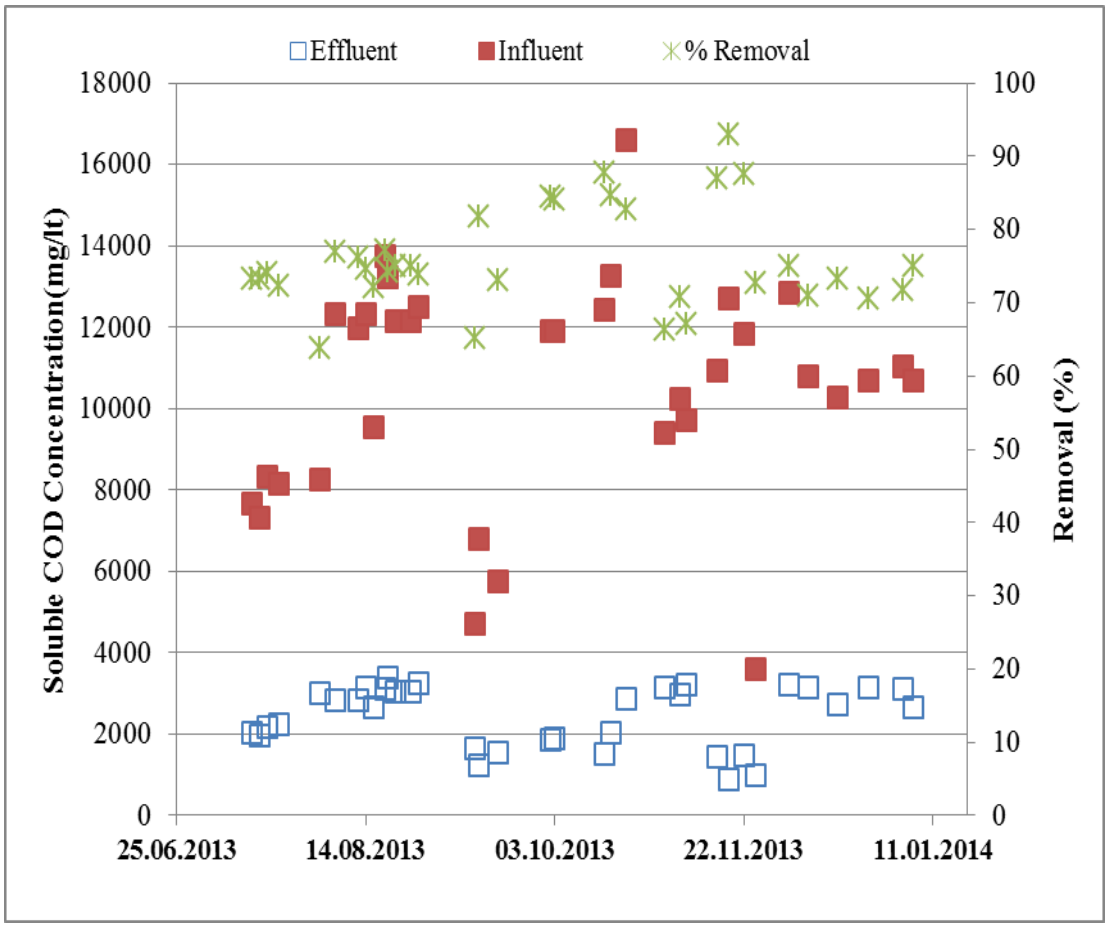

Figure 4.12 : Soluble COD changes in the ASB reactor treating Slurry-II.

3.3.2 TSS and VSS changes

Influent and effluent TSS concentrations were observed as 35309±17691 mg/L and 1095±728 mg/L, respectively (Figures 4.13 and 4.14). Table 4.7 presents the changes in TSS and VSS concentrations in the ASB reactor.

Table 4.7 : TSS and VSS changes in the ASB reactor treating diluted chicken waste**.**

Parameters	Unit	Minimum	Maximum	Mean ± Std. Dev.	Median
TSS $_{influent}$	mg/L	15652	93333	35309±17691	28568
TSS $_{effluent}$	mg/L	215	3735	1095±728	960
TSS removal	%	93	99	96±1.47	97
VSS $_{influent}$	mg/L	7826	56.667	21270±12263	17196
VSS $_{effluent}$	mg/L	105	2025	682±419	580
VSS removal	%	90	99	96±1.98	97

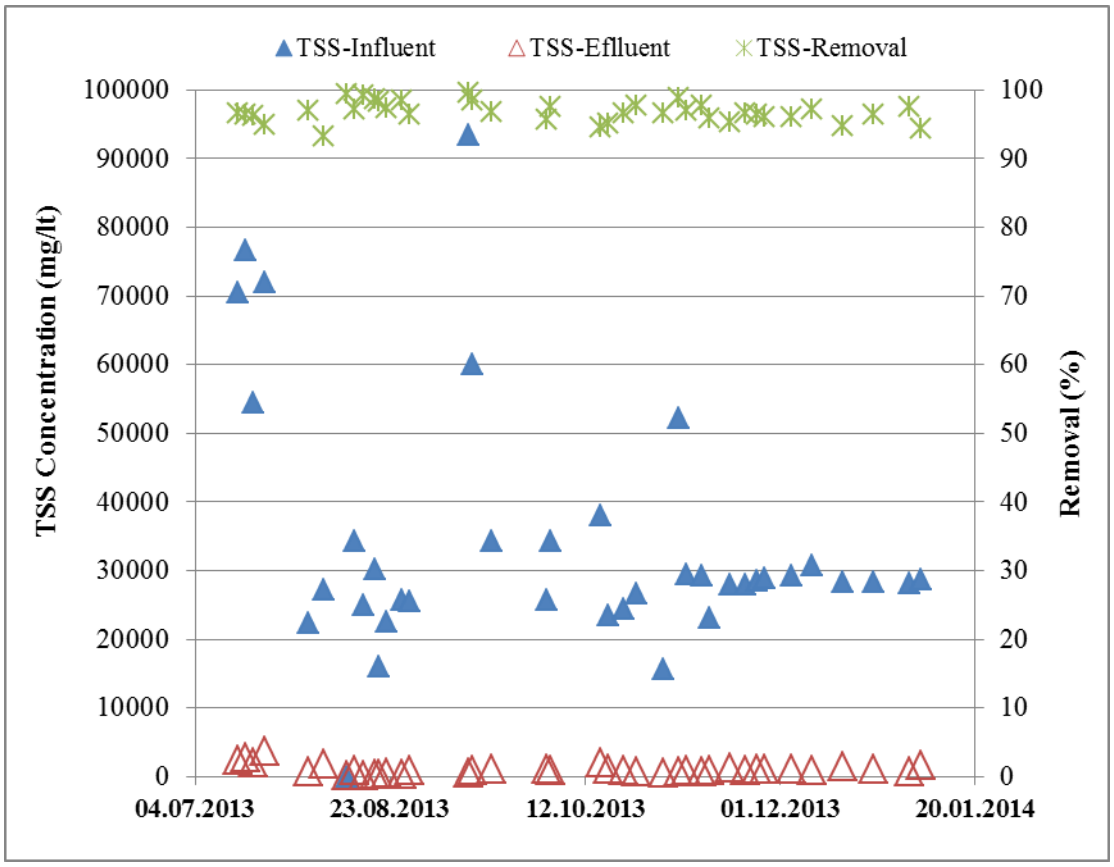

Figure 4.13 : TSS changes in the ASB reactor treating Slurry-II.

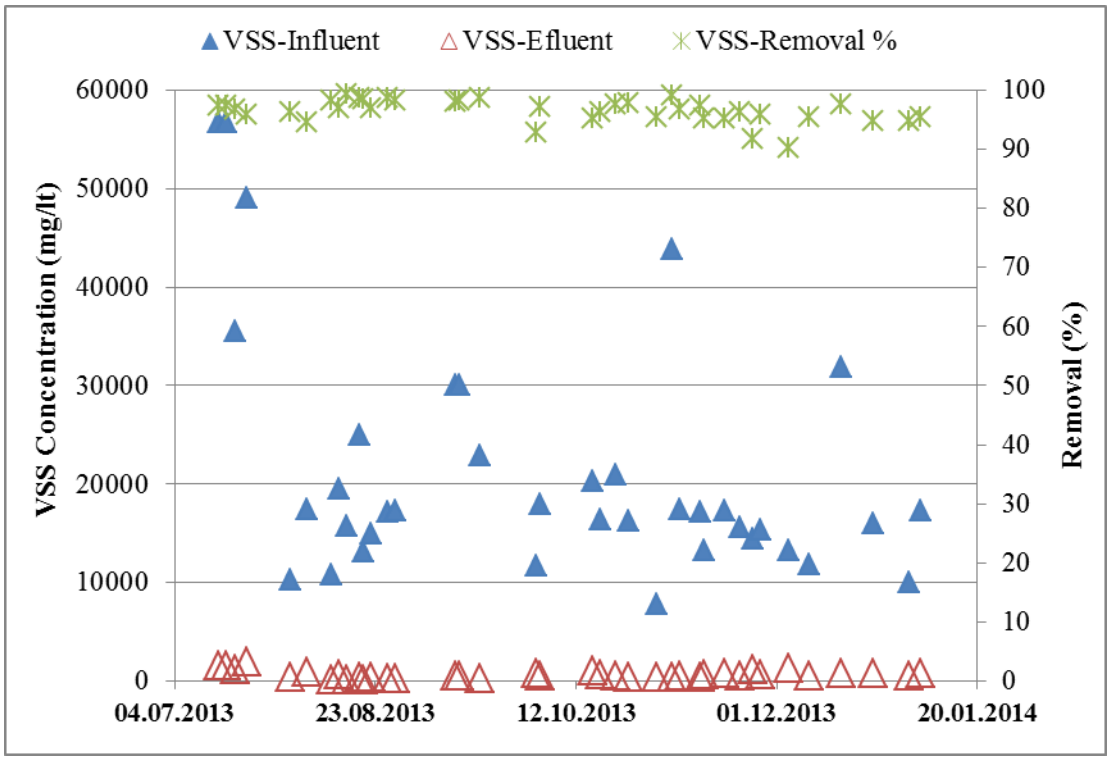

Figure 4.14 : VSS changes in the ASB reactor treating Slurry-II.

3.3.3 pH, alkalinity, and nitrogen changes

Alkalinity results in the influent and effluent were ca. 3285±896 and 1941±604 mg $CaCO_3$/L (Table 4.8). The pH, being a very important parameter in the operation of anaerobic process, was measured 7.62±0.34 and 8.24±0.13 in influent and effluent, respectively. The pH change illustrated in Figures 4.15 and 4.16.

Ammonia nitrogen concentrations in the influent and in the effluent were 1167 and 1152 mg/L, respectively.

Table 4.8 : pH and alkalinity changes in the ASB reactor.

Parameters	Unit	Min.	Max.	Mean ± Std. Dev.	Median
pH $_{influent}$	-	7.04	8.26	7.62±0.34	7.59
pH $_{effluent}$	-	8	8.54	8.24±0.13	8.24
Alkalinity $_{influent}$	mg $CaCO_3$/L	2220	6000	3285±896	2900
Alkalinity $_{effluent}$	mg $CaCO_3$/L	280	2960	1941±604	1980

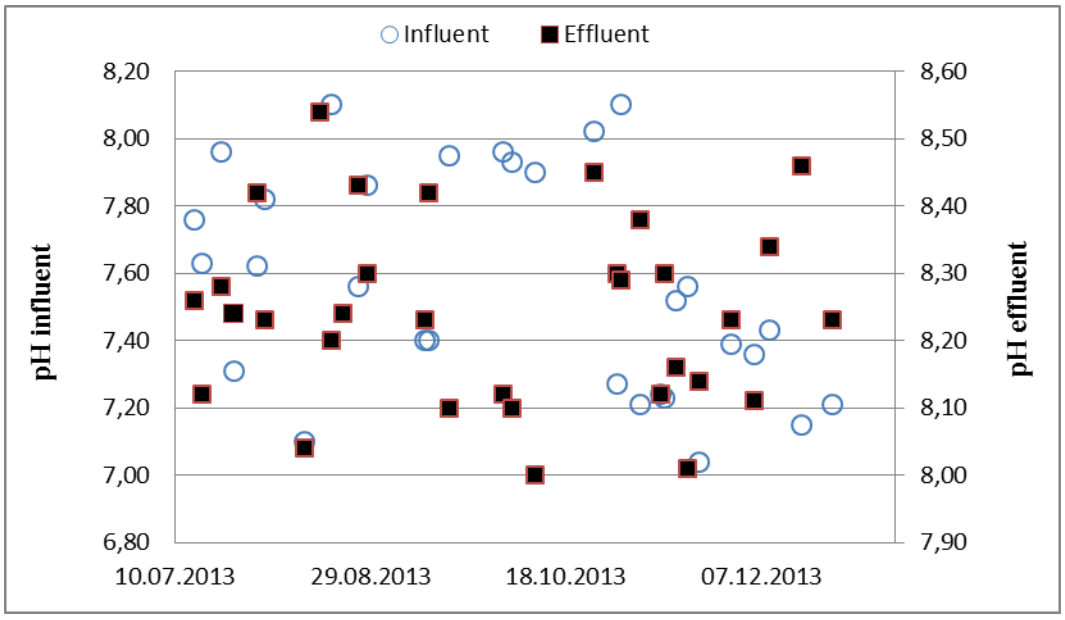

Figure 4.15 : pH changes in the influent in the ASB reactor treating Slurry-II.

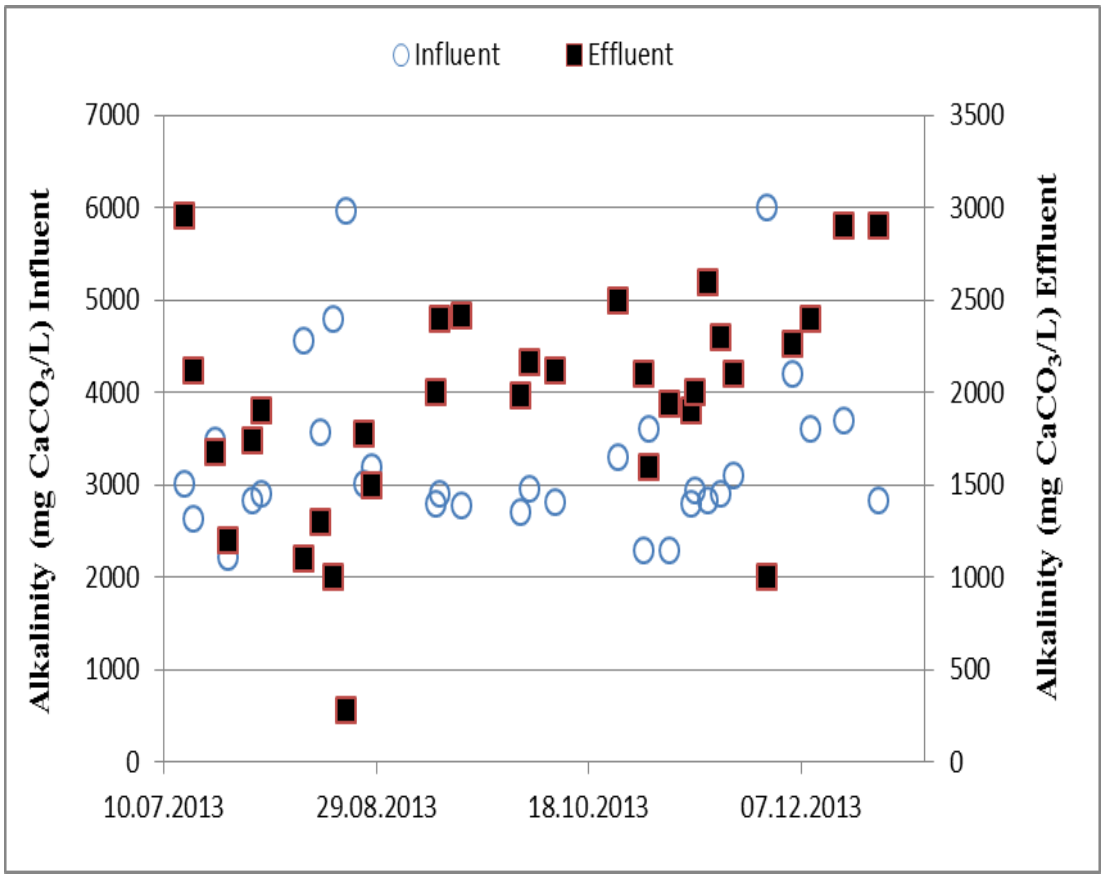

Figure 4.16 : Alkalinity changes in the effluent in the ASB reactor treating Slurry-II.

3.3.4 Biogas production

Recalling that this experiment was a continuation of one that has been started over 3 months ago. Biogas production showed a decline at the beginning of the feeding by Slurry-II, mostly because of the fact that the organic loading rate was increased (i.e. increasing daily feeding volume from 500 ml to 750 ml). Thus, the ASB reactor has been exposed to overloading. Besides, it is clear in Figures 4.17 and 4.18 that the biogas production started to increase immediately again after reducing the organic loading rate. In Table 4.9, daily biogas productions in the ASB reactor fed with Slurry-II are presented.

Table 4.9 : Biogas production in the ASB reactor.

Parameters	Unit	Minimum	Maximum	Mean ± Std. Dev.	Median
Daily biogas	mL/day	0	6.727	1472±1283	1472

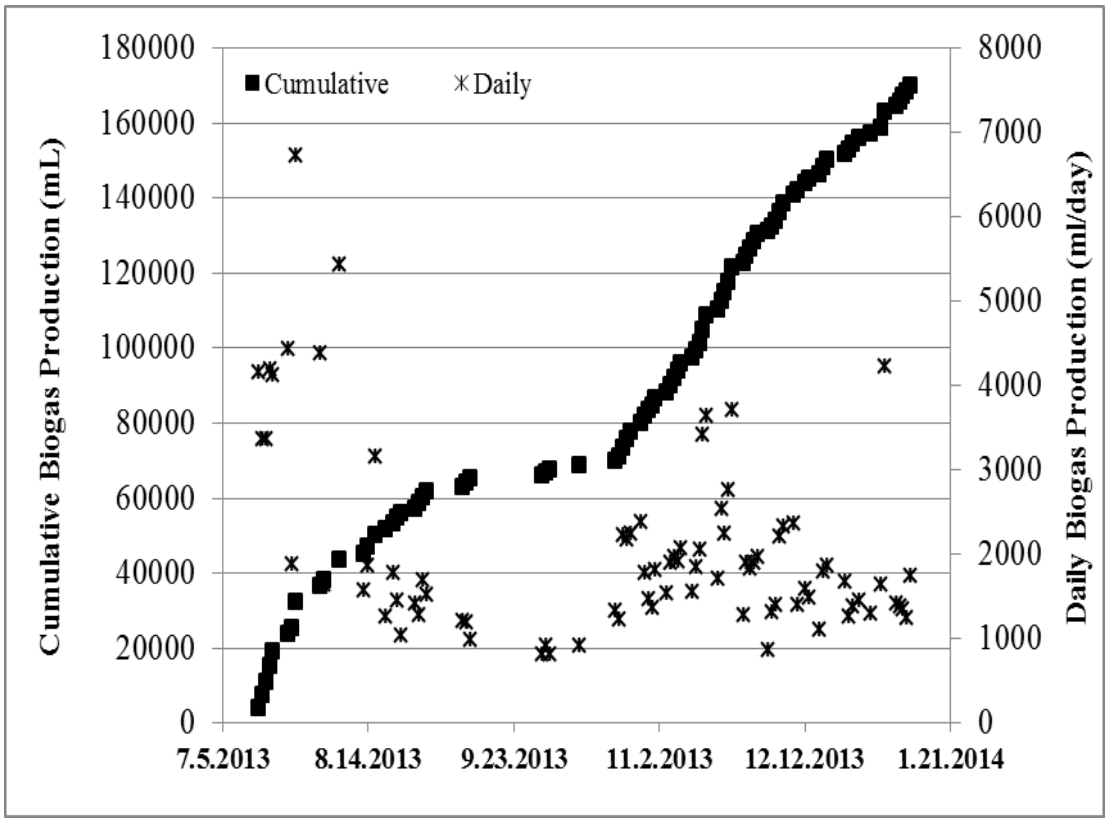

Figure 4.17 : Daily and cumulative biogas productions in the ASB reactor treating Slurry-II.

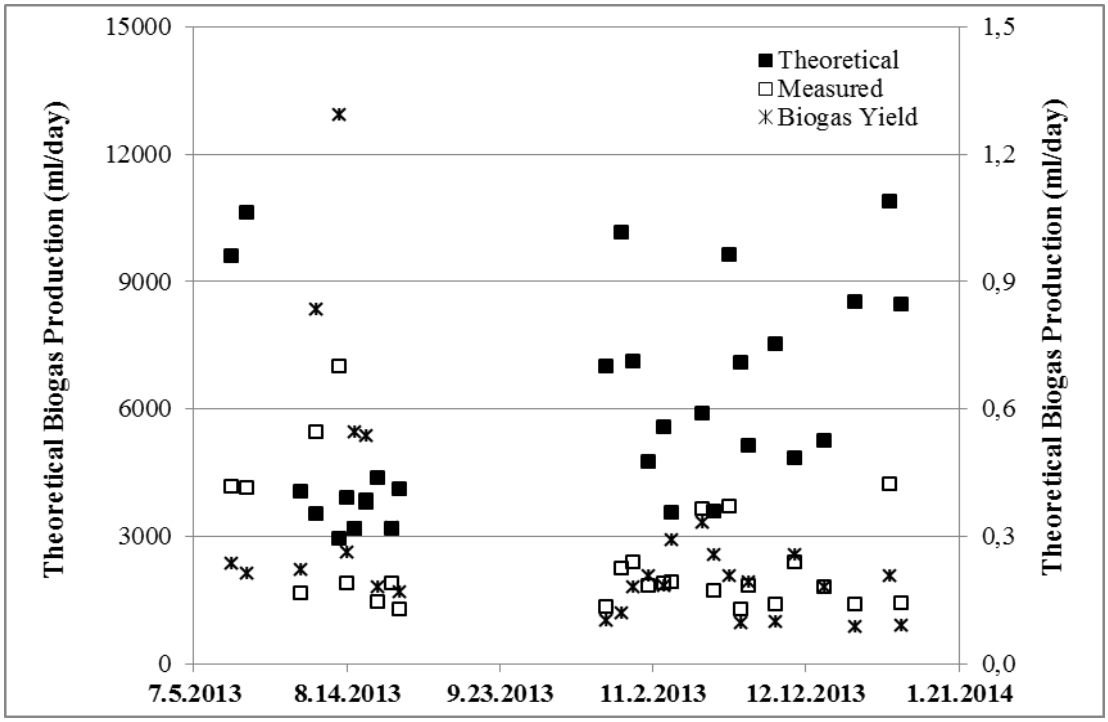

Figure 4.18 : Biogas production and biogas yield per kg total COD removal in the ASB reactor treating Slurry-II.

3.3.5 Biomass and VFA profiles along the ASB reactor

Results of volatile fatty acids (VFA) regarding to the Slurry-II, were also measured from five sampling ports along the ASB reactor and given in Table 4.10.

Table 4.10 : Changes in the VFA concentrations (mg/L) along the ASB reactor for different sampling times.

Port	Acetic	Propionic	Isobutyric	Butyric	Isovaleric	Valeric	Isocaproic	Heptanoic	Formic	Total
Sampling Date: July 22, 2013										
1	1680	70	6	226	51	23	34	12	0	2.107
2	330	22	8	18	10	4	6	0	0	398
3	84	14	5	0	0	0	0	0	0	103
4	313	21	0	17	10	4	6	0	0	371
5	108	3	5	0	0	0	0	0	0	116
Sampling Date: December 4, 2013										
1	1321	97	0	136	61	18	25	14	0	1.672
2	390	26	0	26	15	4	6	0	0	467
3	1548	118	0	164	75	22	31	17	0	1.975
4	105	5	0	5	3	0	0	0	0	118
5	24	0	0	0	0	0	0	0	0	24
Effluent	20	0	0	0	0	0	0	0	0	20

3.3.6 Temperature

The temperature remained almost constant (Figure 4.19) throughout the experiment period and was well maintained within the range of 17-23°C. A reduce in temperature is accompanied with a change of the physical and chemical characterization of the effluent, which can affect design and operation conditions of the treatment system. For example, the solubility of gaseous compounds rises as the temperature decreases lower than 20°C (Lettinga et al., 2001).

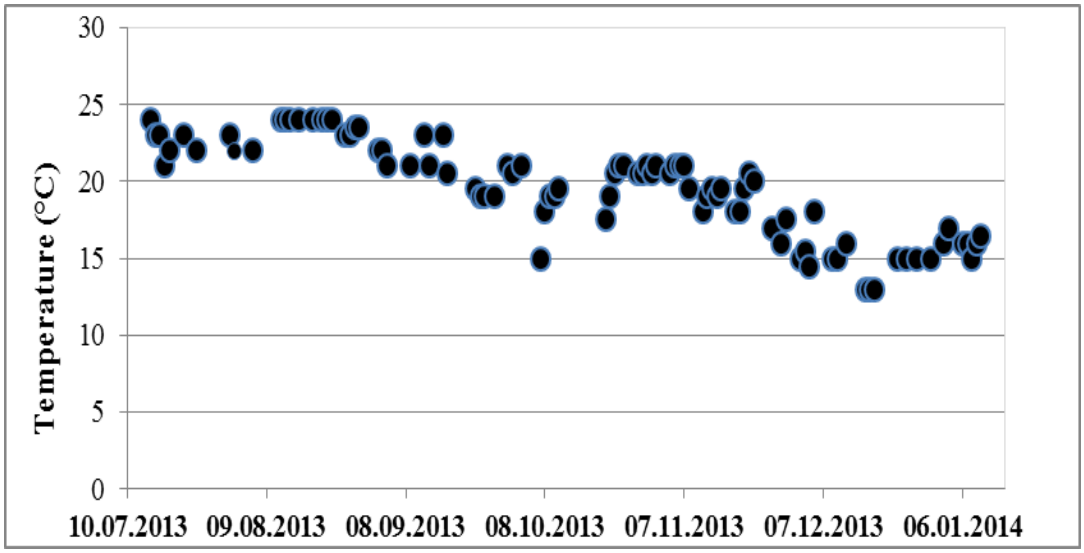

Figure 4.19 : Operating temperature changes inside the ASB reactor treating Slurry-II.

3.4 Microbial quantity along the ASB reactor

Quantitative changes of 16S rDNA and 16S rRNA concentrations in terms of bacteria, archaea and methanogens were determined by Q-PCR. This chapter contains the results and discussion which will be presented as the first and the second sections for Slurry-I (from 30th Mart to 12th July 2013) and Slurry-II (15th July to 10th January 2014), respectively. Two different manure slurries were conducted in this study as described in the Materials and Methods part. The procedures were the same during the whole experimental period with both slurries unless where stated otherwise.

Amount of bacteria, archaea and methanogens were determined by Q-PCR analysis based on 16S rDNA in the samples taken from 5 ports along the ASB reactor for two different sampling times. Quantification results were given in Figures 4.20, 4.21, 4.22. As seen in Figure 4.20a, toward the top of the reactor the number of bacteria decreased from $9.04x10^8$ 16S rDNA copies/ml to $6.8x10^8$ 16S rDNA copies/ml, approximately 24% in the samples taken in May 08, 2013 along the ASB reactor. On the other hand, toward the top of the reactor the number of archaeal and methanogenic community increased from 7x108 16S rDNA copies/ml to $9.8x10^8$ 16S rDNA copies/ml (approx. 28%) and from $3x10^7$ 16S rDNA copies/ml to $7.8x10^7$ 16S rDNA copies/ml (approx. 64%), respectively at the same time.

The numbers in the samples taken in June 12, 2013 are also presented in Figure 4.20. According to Figure 4.20b, bacterial and archaeal results were similar with the results obtained for the first sampling time. Results indicated that while the number of bacterial community decreased along the ASB reactor, the amount of archaea increased. The bacterial 16S rDNA number per ml reduced from $9.74x10^8$ to $6x10^8$ (approx. %38) in a stepwise manner. Meanwhile, archaeal 16S rDNA number/ml increased from $7.9x10^8$ to $1.2x10^9$ (approx. %34). Methanogenic community showed a different pattern which is good correlated (-0.44) between acetate concentration. The number of methanogens displayed an uptrend like archaeal community, however in the second port, the amount of methanogens suddenly decreased from $3.2x10^7$ to $3x10^6$ (approx. %90). Also acetate concentration in the second port increased as 10 fold.

As VFA and microbial analysis of the samples obtained from five specified ports declared, ports 4 and 5 have provided the best activation environment for archaea where the population of archaea increased in correlation with decrement of VFA concentration the fact that previously reported. Acetate amount in samples obtained from these ports was the least. Acetate is directly degraded by acetoclastic methanogens (Shigematsu et al., 2004). Syntrophic association between methanogens and proton-reducing bacteria also converts VFAs to methane (Schnürer et al., 1999). Increasing in methanogens community which clarified by quantitative PCR in all sampling date, therefore was in response to the VFA concentration was (Figures 4.20, 4.21, 4.22).

As discussed in the literature, methanogenesis is the most sensitive step in the anaerobic digestion process and about 70% of methane formed in an anaerobic reactor is derived from acetate (Speece and Parkin, 1983; Gujer and Zehnder, 1983). Hence, an inhibition in the activity of the acetate-utilizing methanogens severely affects the degradation process. A variety of chemicals and environmental conditions may also affect the activity and the composition of methanogens (Ince et al., 2011). Additionally, a negative correlation was found between bacteria-archaea (-0.78) and bacteria-methanogens (-0.6) that also confirms the Q-PCR data obtained along the ASB reactor.

Regarding to the second slurry (Slurry-II) quantification results showed that the number of bacteria decreased from $9.14x10^8$ 16S rDNA copies/ml to $6.2x10^8$ 16S rDNA copies/ml, approximately 32% in the samples taken in July 31, 2013 along the ASB reactor (Figure 4.21). On the other hand, the number of archaeal and methanogenic community increased from $7.8x10^8$ 16S rDNA copies/ml to $11x10^8$ 16S rDNA copies/ml (approx. 41%) and from $3.3x10^7$ 16S rDNA copies/ml to $9.8x10^7$ 16S rDNA copies/ml (approx. 197%), respectively at the same time (Figure 4.21). Methanogenic community showed a good correlation between acetate concentration (-0.65).

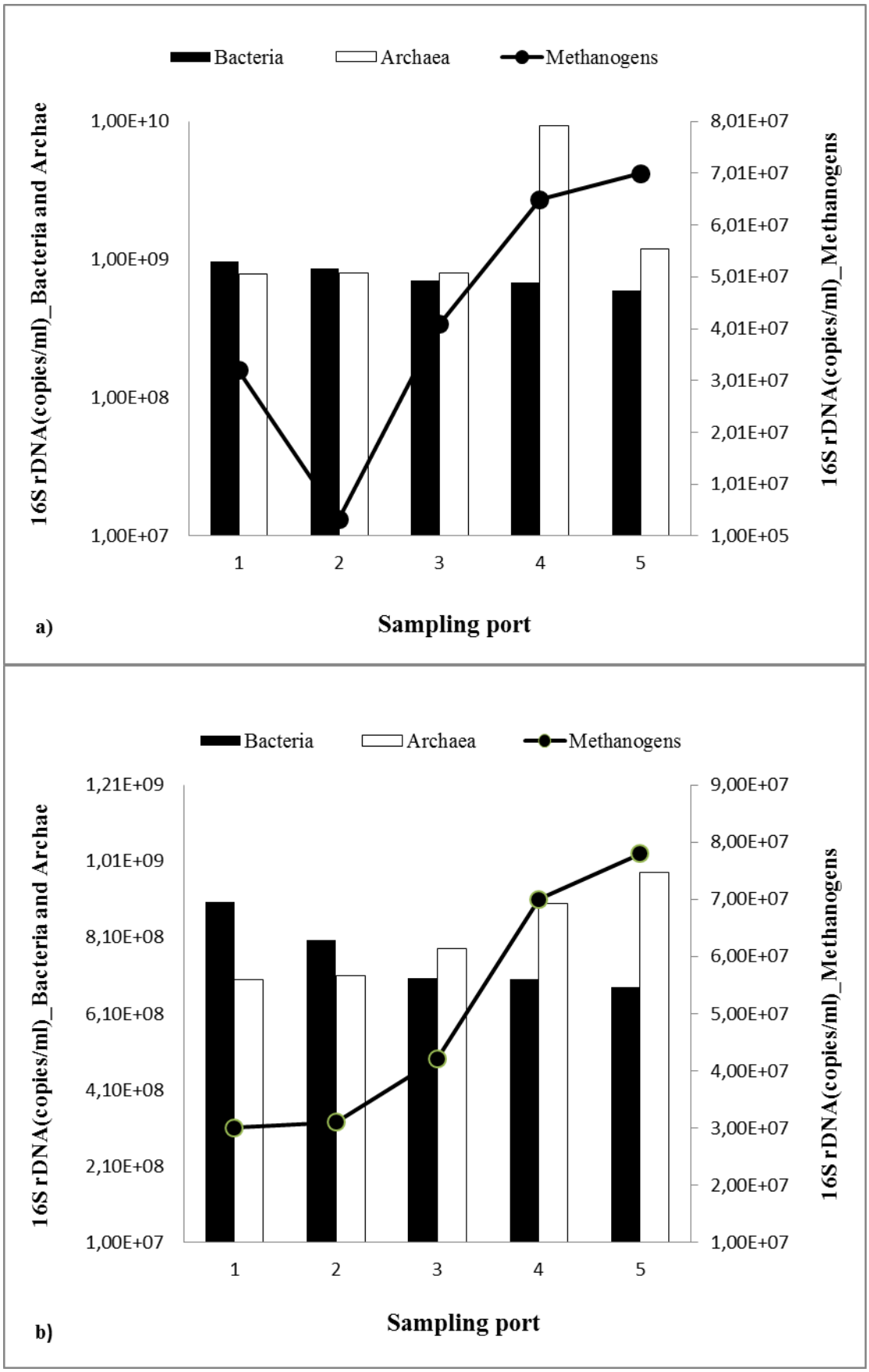

Figure 4.20 : Changes in microbial consortium along the ASB reactor for sampling dates; (a) May 08, 2013 (b) June 12, 2013.

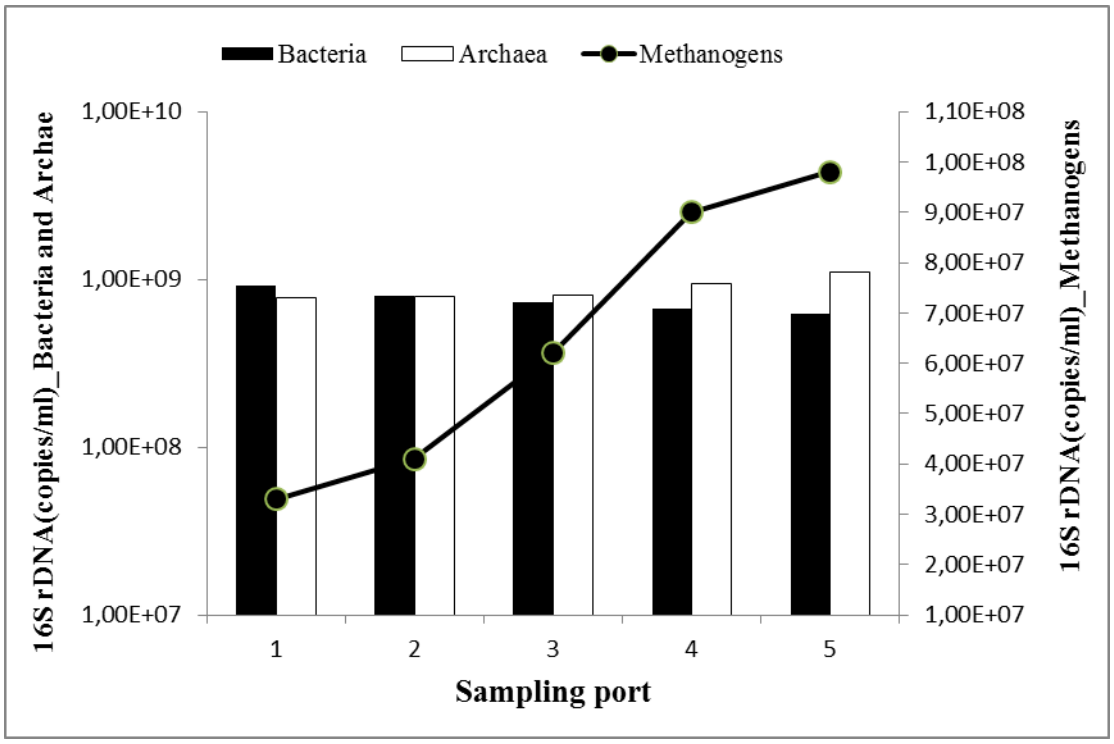

Figure 4.21 : Changes in microbial consortium along the ASB reactor for sampling dates; July 31, 2013.

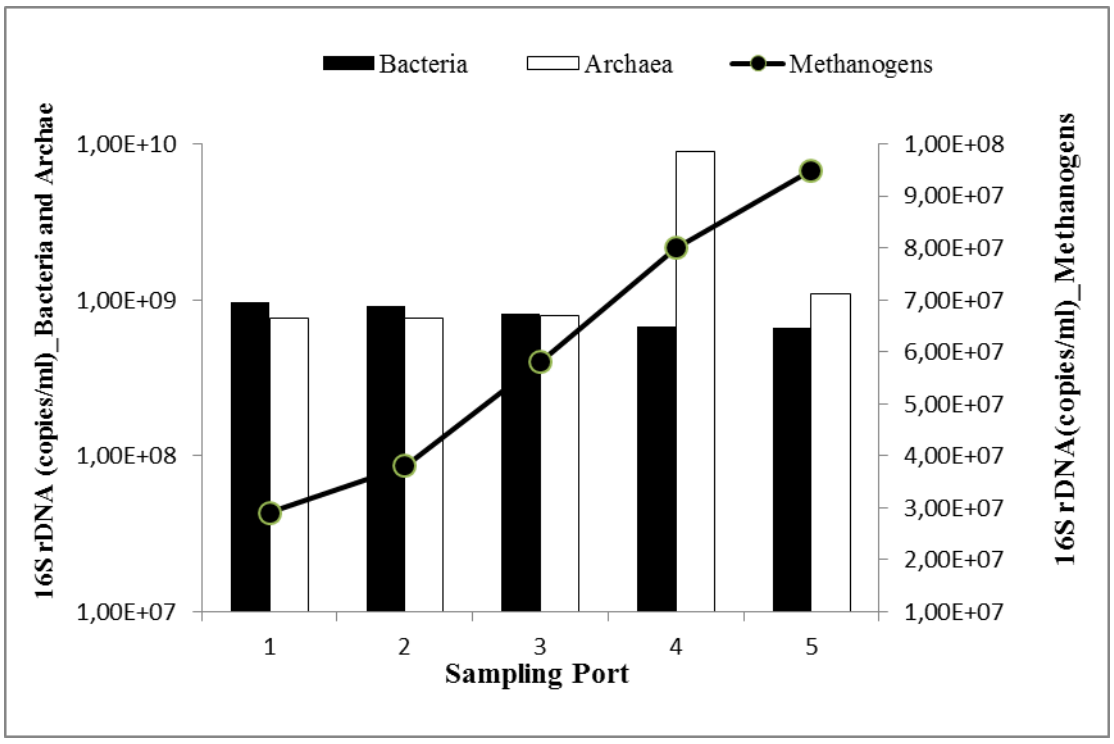

Figure 4.22 : Changes in microbial consortium along the ASB reactor for sampling dates; October 1, 2013.

The numbers in the samples taken in Oct.1, 2013 are also presented in Figure 4.22. According to Figure 4.22, bacterial and archaeal showed a similar changing manner the first sampling time. Similar to the Slurry-I, results indicated that while the number of bacterial community decreased along the ASB reactor, the amount of archaea increased. The bacterial 16S rDNA number per ml reduced from 9.74x108 to 6.6x108 (approx. %32) in a stepwise manner. Archaeal 16S rDNA number/ml on the other hand increased from $7.7x10^8$ to $1.1x10^9$ (approx. %99). The 4th port showed a sudden great amount of increase in archaea community.

As Figure 4.22 shows, methanogenic community also showed a great increase (approx. %228).

Results showed that when the reactor was operating with Slurry-II, the number of methanogens in all five sampling ports displayed an uptrend like archaeal community. Additionally, a negative correlation was found between bacteria-archaea in both sampling dates (-0.94 and -0.52 respectively). Bacteria and methanogens showed a stronger negative correlation in both sampling dates (-0.99 and 0.98 respectively) that also confirms the Q-PCR data obtained along the ASB reactor. Details of methanogens microorganisms in percentage terms are presented in Table 4.11. The percentage number range from 0,70% to 8,91%.

Table 4.11 : Methanogen percentage in archaea.

Ports	08 May2013	12 June 2013	30 July 2013	1 October 2013
1	4,29	4,05	4,23	3,77
2	4,37	0,41	5,19	4,94
3	5,38	5,13	7,65	7,25
4	7,78	0,70	9,47	0,89
5	7,96	5,83	8,91	8,64

5. CONCLUSION AND RECOMMENDATIONS

The poultry and livestock industries are growing rapidly along with the human population. This increased trend in both developed and developing countries yields large quantities of animal waste products.

Anaerobic digestion of livestock manure is an alternative pathway for managing high organic waste loads and its associated problems encountered in big feeding lots and livestock farming. Moreover, anaerobic digestion can result in revenue from energy sales or savings in on-farm energy generation. Results obtained from this study indicated that anaerobic treatment would present a future viable treatment method for poultry industry in case to handling of the produced waste. However, an optimal performance of an anaerobic reactor system could only be achieved by maintaining and controlling some important factors such as pH, temperature, organic loading rate, nutrient requirements, and alkalinity. As observed from the experiment, frequent monitoring is very important for a good performance. On a larger scale, anaerobic treatment technology has the potential for energy savings if used instead of aerobic treatment. In this study, a successful anaerobic treatment of the diluted chicken manure by a lab-scale ASB reactor inoculated with the granular seed was realized at ambient operating temperatures.

Result from these study showed that the pH condition throughout the experiments with Slurry-I and Slurry-II was within the pH range (6.5 – 7.5) that has been reported as the optimum condition for most anaerobic systems. Hence, during all period pH was not a problem and was not the cause of failure.

It was observed that the Total COD removal efficiencies on average were 89% and 90% in case with Slurry-I and Slurry-II, respectively. Despite of some deficiencies and drawbacks (i.e. unavoidable biogas loss and overloading) during the experiments, still high COD removals and daily biogas generations could be obtained.

Moreover, anaerobic treatability of chicken manure could be achieved with more feasible solution in this study due to the fact that external heating requirement up to

mesophilic condition (35°C) was avoided. With the all experiment duration at ambient temperature, microorganisms appeared to be highly resilient to temperature changes in the ASB reactor.

Although the ASB reactor was operated at room temperature, results still indicated effective performances in terms of average Total COD removals (ca. 89 and 90%) and average CH_4 production rates (ca. 0.21 and 0.18 m^3 per kg of COD removed) for Slurry-I and Slurry-II, respectively at a HRT of about 13 days. On the other hand, the ASB reactor did not indicate any inhibition due to dilution of the raw chicken manure during the operating period of this study, since it is reported that the inhibition regarding free ammonia might be distinct especially when digesting raw poultry manure. The necessity to comply with discharge limits has become a matter of increasing concern to poultry industry. Hence, an effective post-treatment unit is required for the anaerobically pretreated diluted poultry manure for environmental protection.

In this study, the microbial quantification results indicated a shift in methanogenic community during biogas recovery in the ASB reactor using 16S rDNA-based Q-PCR. Microbial quantification results for Slurry-I and Slurry-II indicated that as the number of bacterial community decreased, the amount of archaea increased through the effective digestion volume of the ASB reactor. Moreover, the number of methanogens displayed an uptrend like archaeal community and a strong correlation as -0,44 ($p<0,005$) and -0.65 ($p<0,005$) for Slurry-I and Slurry-II, respectively, was found between methanogenic community and acetate concentration.

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LIST OF PUBLICATIONS

- Jaxybayeva A., Yangin-Gomec C., Cetecioglu Z., Ozbayram E. G., Yilmaz F., İnce O., "Bioenergy production from diluted poultry manure and microbial consortium inside Anaerobic Sludge Bed Reactor operated at sub-mesophilic conditions", Journal of Environmental Science and Health B (Pesticides, Food Contaminants & Agricultural Wates), 2014, (in press).

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